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Algal Uptake Of Uranium, Barium, Cobalt, Nickel And Vanadium: Studies Of Natural And Experimental Systems

Henrietta Mann

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ALGAL UPTAKE OF U, Ba, Co, Ni AND V: STUDIES OF NATURAL AND EXPERIMENTAL SYSTEMS

by

Henrietta Mann

Department of Geology

Submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
London, Ontario

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ABSTRACT

Monocultures of freshwater green algae Ankistrodesmus sp. and Selenastrum sp. cultured in dilute solutions of metals chelated with EDTA, revealed massive uptake of U and Ba. For 2 ppm metal-spiked TBIM culture media at pH 8.1-8.2 and growth durations of 3-21 days at 13-16°C, both species accumulate U and Ba at levels of 1,000 to 10,000 ppm by dry weight, representing a concentration factor of up to 5000 times the starting aqueous metal solute concentration. Uptake was comparable for passage of 100 ml spiked culture media through algal mats over 100 min. For 2 ppm Co-, Ni- and V-spiked culture media, algal uptake was 40-600 ppm Ni, 17 ppm Co and 12 ppm V, respectively. At aqueous solute concentrations of 40 and 20 ppb the algae absorbed 3,000-6,000 ppm U and Ba, giving concentration factors up to 200,000. The lowest Ba aqueous concentration was about equivalent to, and the U concentration 100 times, natural fresh water abundances.

Examination of Thames River waters and selected micro-organisms of its aquatic ecosystem revealed an average dissolved U of 1.46 ppb $\pm$ 0.61 1σ, representing about twice the global mean riverine solute concentration of 0.6 ppb U. Suspended particulates, principally algae, contain U at 10^3 to 10^5 times that of their river habitat, averaging 28,300 ppb U: algae carry about 15% of the total riverine U flux. Seasonal variations of dissolved and

algal uranium occur, peaking over the fall and winter months September through February. Winter and fall peaks correlate with higher discharge rates during thawing and rainfall maxima respectfively. One community of the filamentous algae Spirogyra sp. contained intracellular crystals tentatively identified as the Ca-oxylate weddelite.

At Elliot Lake, tailings waters contain high concentrations of dissolved uranium along with other heavy metals, and spectacular growths of Euglena sp. In natural drainage waters dissolved U averages 0.23 $\pm$ 0.19 ppb 1σ, whereas tailings effluent averages 85 $\pm$ 62 ppb 1σ at the Nordic Main, 220 $\pm$ 230 ppb 1σ for Westarm and 273 $\pm$ 215 ppb 1σ for the Stanrock impoundment. Suspended micro-organisms, chiefly algae, in natural waters contain an average 19,300 ppb U, and up to 1,200,000 ppb or 1.2% of the cell by dry weight. Waterborne suspended particulates (mostly algae, 11.2 $\pm$ 21.3 1σ mg/litre) carry 216 ng U/litre, or about 45% of the total riverine uranium flux. The average partitioning of uranium between micro-organisms and waters of the drainage system is 8×10^4, close to that of 2×10^4 for Thames River waters. Euglena communities thriving in tailings waters contain an average 3×10^5 ppb U, in addition to 40 wt. % Fe, Al (14,000-40,000 ppm), Ba (7-90), Co (250), Cu (70-40), Mn (200-500), Ni (80-200), Pb (1300-2,000), Si (200-2,000), Sr (50-200), Ti (400-900), V

(30-70) and Zn (50-230 ppm), as well as significant Ag, Be, Th and Zr. A reconaissence survey of algae from selected freshwater and marine environments revealed high concentrations of many elements in addition to U, including Ag, Al, Ba, Be, Co, Cr, Cu, Fe, Mn, Ni, Pb, Si, Sr, Th, Ti, V, Zn, and Zr.

Given the abundance of algae in both fresh and marine waters, coupled with the known association of plankton with U-rich Black Sea muds, these results may indicate that algae play a significant role in mediating transfer of U and Ba, as well as many other elements from the hydrosphere to sedimentary reservoirs.

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CHAPTER 1

INTRODUCTION AND STATEMENT OF THE PROBLEM

1.1 Biogeochemical cycling of the elements - context

A fundamental problem of geochemistry is that of understanding transport and precipitation mechanisms of elements in the hydrosphere and the mechanisms of their incorporation into sediments (Goldschmidt, 1937, 1954). Such processes may in part be biologically mediated, and concentration factors for chemical elements in organisms of up to 250,000 over their marine aqueous abundance have been measured for iron, manganese and lead (Fairbridge, 1972; Trudinger, 1976).

In a discussion of microbiological processes related

to the genesis of ore deposits Trudinger (1976) listed four specific ways in which microorganisms contribute to metal accumulations. These are:

1. Direct concentration of metals from dilute solutions by micro-organisms.
2. Modification of the physico-chemical conditions in the environment. For instance the various respiratory and fermentative reactions carried out by micro-organisms may exert a control on pH and Eh, as well as concentrations of chemical species such as CO_2 or H_2S, which in turn mediate metal release or fixation.
3. Generation of organic matter. Because of its complexing properties organic matter has a profound influence on the mobilization, transport and fixation of metals.
4. Direct catalysis of oxidation-reduction reactions which act to solubilise or fix metals. Iron and manganese oxidizing bacteria are well known examples of this process.

This thesis is specifically concerned with the first process.

Microorganisms in general possess the ability to accumulate many elements from solutions with very low concentrations. Trudinger (1976) lists element concentrations in marine microbiota, which consist largely of algae and bacteria, representing up to 270,000 times their abundance in seawater.

A number of elements which are extracted by living organisms from the environment have essential physiological functions, being components of cellular structures or of enzymic catalysts involved in energy production or cell synthesis. For other elements however, no physiological role has yet been identified, and the accumulation of these elements may reflect the strong complexing character of the organic components of the living cell, or the macromolecules of its degradation.

The classic examples of extensive mineral deposition resulting from microbial accumulation of elements are the silica and carbonate deposits resulting from massive sedimentation of silicious skeletons of diatoms and radiolaria, and of calcium carbonate tests by corals, etc. The scale of this biologically mediated process is evident from the fact that about 6×10^{23} g of Si is removed from the oceans per billion years, representing a significant fraction of the entire mass of the continental crust (18.8×10^{24} g). The scale and variety of biomineralization have been emphasized in a review by Lowenstam (1981) and the effect of such bio-precipitants on the global cycling of elements between geochemical reservoirs by Fyfe (1974, 1979, 1981).

Whereas the role of microbiological activity in the output of major dissolved species including Si, Ca and P from the hydrosphere is known in broad terms, this is generally not the case for the interaction of micro-

organisms with dissolved trace elements. However, in a series of classic studies Beveridge and Murray (1976, 1980) clearly demonstrated the uptake and retention of over forty elements by bacteria. It is axiomatic that organic rich rocks, such as black shales, coals and petroleum, sporadically contain a distinctive complement of heavy metals, sometimes greatly enriched over their crustal average (Vine and Tourtelot, 1970; see Meloche, 1982). It is this association, providing indirect evidence for the participation of organisms in metal concentration, that provides the rationale for direct studies of metal uptake by living organisms.

In view of the above and the general lack of information on metal uptake by microorganisms other than bacteria, this thesis was designed to examine the role of algae in extracting metals from the hydrosphere. Algae are ubiquitous in both fresh and marine waters; their small size and large mass, up to 19 x 10^6 cells per ml (Steinhorn and Gat, 1983), make them a potent factor in microbiological cycling of the elements. A comprehensive account of fossil algae is given by Flugel (1977).

This thesis covers three specific areas. First, a series of experimental studies designed to examine the uptake of Ba, Co, Ni, V and U from dilute solutions containing the chelating agent EDTA, by two freshwater species of algae, namely Ankistrodesmus sp. and Selenastrum

capricornutum. A number of treatments were employed, where the influence of exposure times to metals, and of varying the dissolved metal abundance was explored. These experiments are reported in chapter 2.

The second and third parts involved examination of uranium abundance in river waters and microorganisms of the aquatic ecosystems of the upper Thames river (chapter 3) and near Elliot Lake (chapter 4). Whereas the former is largely a natural drainage system, acid tailing waters at Elliot Lake, containing high concentrations of uranium along with other heavy metals, provides the opportunity to examine the response of microorganisms to such elevated levels of toxic metals. In view of the emphasis on algae in biogeochemical cycling of elements, chapter 1 contains a brief synthesis of taxonomic and structural information on the Chlorophycophyta (the green algae), to which both of the algae used in experiments belong.

Chapter 5 summarises results from the first three parts collectively, along with data for algae from other selected freshwater and marine habitats. Information from possible natural experiments, namely the distinctive heavy metal complement of algal derived crude oils, is synthesised, and compared to results obtained in these studies. The final chapter concludes with a brief discussion of the role played by algae in trace element cycling.

1.2 General features of the division Chlorophycophyta (green algae)

The green algae, Chlorophycophyta, is one of the major groups of algae given both the abundance of their species and genera, as well as the frequency of their occurence (Table 1.1). Chlorophycophyta grow in waters of a great range of salinity, varying from oligotrophic freshwaters to those that are marine or supersaturated with solutes (Bold and Wynne, 1978). A number of species grow in brackish waters. Several orders of green algae are exclusively marine. Both benthic and planktonic species occur; a number grow in subaerial habitats.

A great range of organization of the plant body occurs in the Chlorophycophyta including unicellular (Ankistrodesmus, Selenastrum), colonial (Pediastrum), filamentous (Draparnaldia, Spirogyra), membranous (Monostroma), and tubular types (Enteromorpha). Examples of the first three varieties are illustrated and tabulated at appropriate junctures in chapters 2 and 3.

Algae can float, swim or be attached and stationery. Cells contain plastids (chloroplasts) in which chlorophyll (grass-green in colour) is predominant and in which there is usually a shiny, starch-storing body, termed the pyrenoid (Fig. 1.1). Pigments are chlorophyll-a, chlorophyll-b, 2-3 carotenes, as many as 6 or possibly 10 xanthophylls, and with red carotenoids (haematochrome) sometimes

Table 1.1 Summary of some algal divisions and their more significant characteristics (modified after Bold & Wynne, 1978).

Division	Common Name	Pigments and Plastid Organization in Photosynthetic Species	Stored Food	Cell Wall	Flagellar Number and Insertion	Habitat
Cyanochloronta or Cyanobacteria*	Blue-green algae	Chlorophyll a; C-phycocyanin, allophycocyanin-C-phycoerythrin; ß-carotene and several xanthophylls	Cyanophycean granules (arginine and aspartic acid); polyglucose (glycogen-like)	α, ε-Diaminopimelic glucosaemine, alanine, etc.	Absent	fw,bw, sw, t
Chlorophycophyta	Green algae	Chlorophyyl a, b; α-, ß-, and γ-carotenes + several xanthophylls;	Starch (amylose and amylopectin) (oil in some)	Cellulose in many (= ß -1,4-glucopyranoside), hydroxyproline glycosides; xylans and mannans; or wall absent; calcified in some	1,2-8, many, equal apical	fw, bw, sw, t
Charophyta	Stoneworts	Chlorophyll a, b; α-ß-, and γ-carotenes + several xanthophylls; thylakoids variably associated	Starch resembling that of land plants	Cellulose (= ß -1,4-glucophyranoside); some calcified	2, equal, subapical	fw, bw
Euglenophycophyta	Euglenoids	Chlorophyll a, b; ß-carotene + several xanthophylls; 2-6 thylakoids/stack, sometimes many	Paramylon (= ß -1,3-glucopyranoside), oil	Absent	1-3(-7) apical, subapical	fw, bw, sw, t
Phaeophycophyta	Brown algae	Chlorophyll a, c; ß-carotene + fucoxanthin and several other xanthophylls; 2-6 thylakoids/stack	Laminaran (= ß -1,3-glucopyranoside, predominantly); mannitol	Cellulose, alginic acid, and sulfated mucopolysaccharides (fucoidan)	2, unequal lateral	fw (very rare), bw, sw
Chrysophycophyta	Golden and yellow-green algae (including diatoms)	Chlorophyll a, c(c lacking in some); α-ß-, and ε-carotene + several xanthophylls; including fucoxanthin in Chrysophyceae, Bacillariophyceae, and Prymnesiophyceae; 3 thylakoids/stack	Chrysolaminaran (ß-1,3-glucopyranoside, predominantly); oil	Cellulose, silica, calcium, carbonate mucilaginous substances, and chitin; or wall absent	1-2, unequal or equal apical	fw, bw, sw, t
Pyrrhophycophyta	Dinoflagellates	Chlorophyll a, c; ß-carotene + several xanthophylls; 3 thylakoids/stack	Starch (oil in some)	Cellulose or absent; mucilaginous substances	2, one trailing, one girdling	fw, bw, sw
Cryptophycophyta	Crytomonads	Chlorophyll a, c; α-, ß-, and ε-carotene; distinctive xanthophylls (alloxanthin, croco xanthin, monado-xanthin); phycobilins; 2 thylakoids/stack	Starch	Absent	2,unequal subapical	fw, bw, sw
Rhodophycophyta	Red algae	Chlorophyll a (d in some Florideophycidae); R- and C-phycocyanin, allophycocyanin; R- and B-phycoerythrin. α- + ß-carotene + several xanthophylls; thylakoids single, not associated	Floridean Starch (glycogen-like)	Cellulose, xylans, several sulfated polysaccharides (galactans) calcification in some	Absent	fw (some), bw, sw (most)

* According to Rippka et al. (1979), and Margulis and Schwartz (1982).

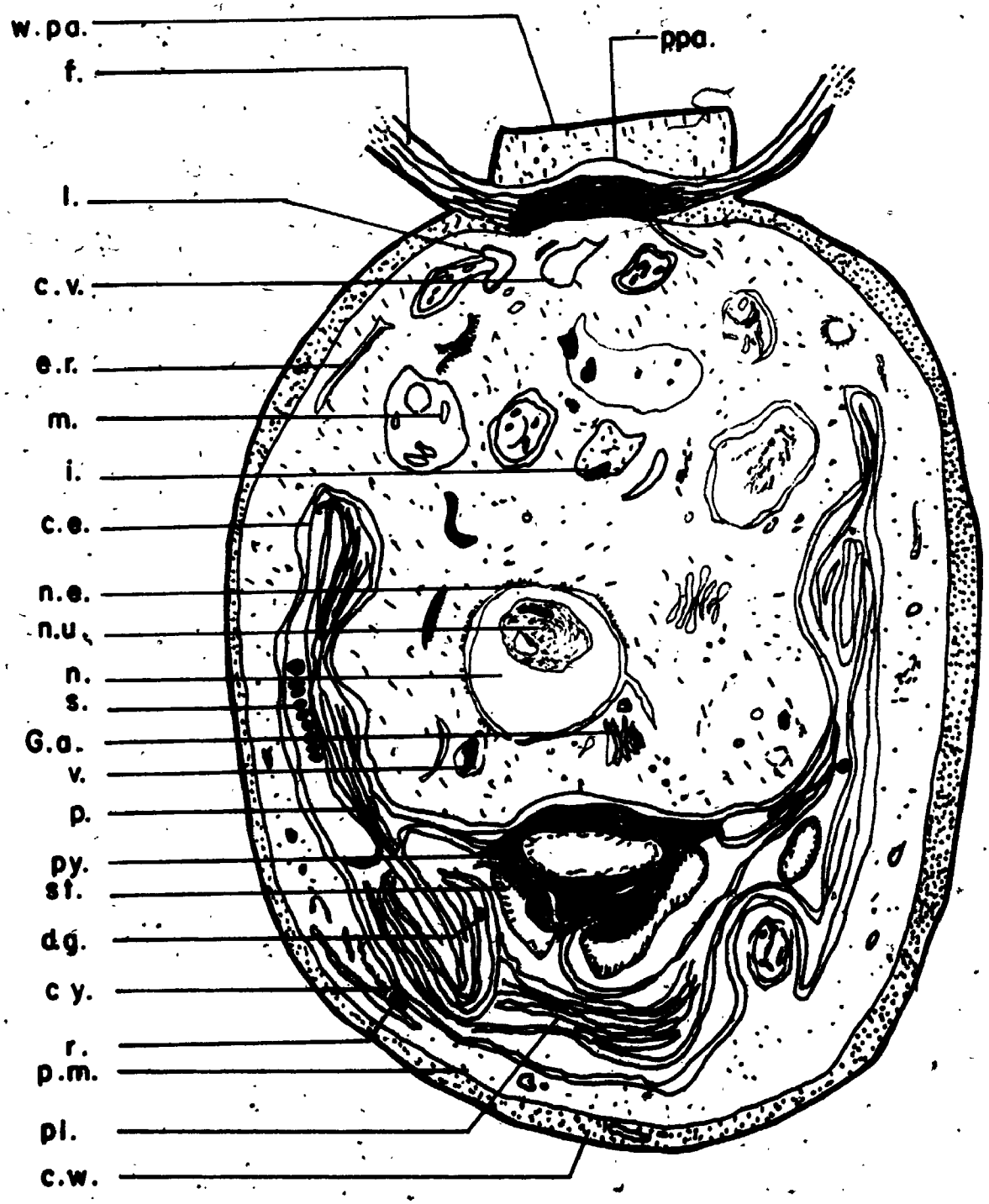
fw = freshwater

bw = brackish water

sw = salt water

t = terrestial (soil, rocks, etc.)

Fig. 1.1 General structure of unicellular algae. c.e., chloroplast envelope; c.v., contractile vacuole; c.w., cell wall; cy., cytoplasm; d.g., dense granule; e.r., endoplasmic reticulum; f., flagellum; G.a., Golgi apparatus; i., inclusion; l., lipid body; m., mitochondrion; n., nucleus; n.e., nuclear envelope; nu., nucleolus; p., plastid; p.pa., plasma papilla; pl., chloroplast; p.m., plasma membrane; py., pyrenoid; r., ribosomes; s., stigma; st., starch; v., vesicle; w.pa., wall papilla. After Bold and Wynne (1978), Fig. 3.7.


w.pa.
ppa.
f.
l.
c.v.
e.r.
m.
i.
c.e.
n.e.
n.u.
n.
s.
G.a.
v.
p.
py.
sf.
d.g.
c y.
r.
p.m.
pl.
c.w.

present. For intracellular starch the iodine test is positive (Table 1.1). Cell walls are composed of cellulose and pectin. Swimming cells or mobile reproductive elements are furnished with 2, 4, or (rarely) as many as 8 flagella of equal length, attached to the anterior end. Sexual reproduction is by iso-, amiso-, and by heterogametes (Prescott, 1978).

Cellular organization in the Chlorophycophyta, as in all algae except the Cyanochloronta (Cyanobacteria) is eukaryotic. The cells are for the most part uniceleate, but the multinucleate condition characterizes several orders and occurs among certain genera of Chloroccales (Bold and Wynne, 1978).

CHAPTER 2

ALGAL UPTAKE OF U, Ba, Co, Ni AND V FROM DILUTE SOLUTIONS: AN EXPERIMENTAL STUDY

2.1 Introduction

As stated in chapter 1, there is little quantitative information on the extent of uptake of many trace metals by algae. The studies reported here were designed therefore to examine the uptake of Ba, Co, Ni, V and U from dilute solutions with the chelating agent (EDTA) by the freshwater species of green algae (Ankistrodesmus sp. and Selenastrum capricotum). Uranium was selected to provide an experimental counterpart to studies on the interaction of aquatic microorganisms with uranium enriched tailings waters, as

Table 2.1. Summary of objectives and experimental conditions for determining the uptake of specified metals by algae.

Experiment number	Objectives	Conditions	Culture	Metals	Concentration
0	Establishing growth rates and conditions	40 days in TBIM	Ankistrodesmus Selanastrum	-	-
1	Determine uptake or toxicity	algae growing 5-25 days in spiked solution	Ankistrodesmus Selanastrum	U, Ba, Co Ni, V	2 ppm
2	Determine uptake or toxicity	algae in mat, rapid single pass of solutions	Ankistrodesmus	U, Ba, Co; Ni, V	2 ppm
3	Determine uptake or toxicity	algae in mat - slow pass of spiked solution	Ankistrodesmus	U, Ba, Co, Ni, V	200 ppb 2,10 ppm
4	Determine uptake or toxicity	algae growing 5-25 days in spiked solution	Ankistrodesmus	Co, Ni, U, Ba, U	40 ppb 20 ppb
5	Determine uptake on dead cells	dead cells, rapid pass of spiked sols.	Ankistrodesmus	U, Ba	2 ppm
6	Determine uptake - U levels close to natural H_2O's	algae - natural cultures in aquarium	Natural cultures	U	0.06 ppb
7	Uptake sites on cells, Analytical SEM	algae growing in spiked sols. 20 days	Ankistrodesmus	U, Ba	5,2 ppm

Plate 2.1 SEM micrograph of Selenastrum capricornutum.

A. Population contaminated by bacteria.

Magnification 5,000 diameters.

B. Selenastrum culture, magnification 8,000 diameters.

A

B

Plate 2.2 SEM micrograph of Ankistrodesmus sp. culture.

A. Magnification, 3,000 diameters.

B. Magnification, 5,000 diameters.

A

B

described in chapter 4. Vanadium was also utilized given its congruent behaviour with uranium during oxidation. Uptake of Ba, Co, and Ni by bacteria has been reported by Beveridge (1978) and Beveridge and Murray (1976, 1980); these elements were employed in order to assess their uptake by algae. A number of different experimental conditions, or treatments, were utilised, in the context of possible variations in natural conditions; these include metal exposure to live and dead cells, algae cultured in spiked media over long durations versus rapid single pass exposure, the presence of single or mixed metals, and metal solute concentrations spanning nearly four orders of magnitude. Of these four variables, the first was employed to evaluate active versus passive uptake by algae; the second to explore the kinetics of metal uptake; the third, parameter to involve the competition of various metals for uptake by algae; and the fourth monitors uptake behaviour over a range of metal solute concentrations spanning levels near toxicity, down to those approaching natural conditions. These experimental designs are summarized in Table 2.1, and referred to hereafter as experimental series 1, 2, etc.

2.2 Materials and Experimental Methods

2.2.1 Experimental Series - 1

Species of algae (Ankistrodesmus and Selenastrum) were obtained from the Ontario Ministry of the Environment,

Table 2.2. Tris-buffered inorganic medium (TBIM)

Chemical compound	Amount	Final concn., μM
KNO_3	20 ml of 0.1 M solution	2000
Na_2HPO_4	10 ml of 0.1 M solution	1000
$MgSO_4.7H_2O$	3 ml of 0.1 M solution	300
$CaCl_2.2H_2O$	1 ml of 0.1 M solution	100
Tri(hydroxymethyl)-aminomethane (TRIS)	25 ml of 0.2 M solution	5000

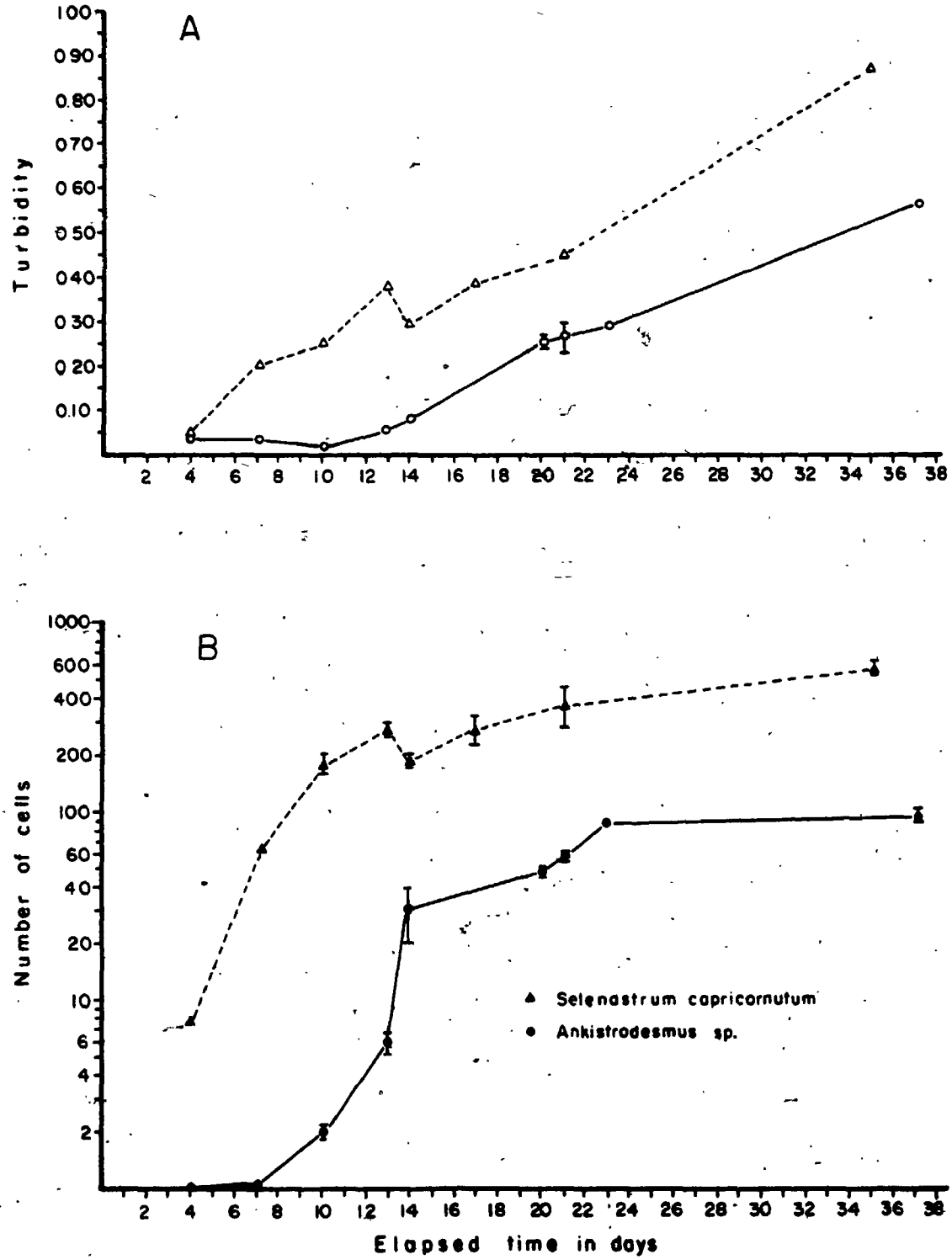
Each of the above is added to approximately 800 ml deionized water. One milliliter of each of the following micronutrient stock solutions is then added and a final dilution of 1 liter made.

I.	EDTA	50 g per liter deionized water		170
	KOH, 85%	31 g per liter deionized water		470
II.	H_3BO_3	11.42 g per liter deionized water		185
III.	$FeSO_4.7H_2O$	4.98 g per liter acidified water*		17.8
IV.	$ZnSO_4.7H_2O$	8.82 g	per liter acidified water*	30.0
	$MnCl_2.4H_2O$	1.44 g		7.3
	$Na_2.MoO_4.2H_2O$	1.19 g		
	$CuSO_4.5H_2O$	1.57 g		6.3
	$Co(NO_3)_2.6H_2O$	0.49 g		1.7

* Acidified water: 999 ml deionized water, 1 ml concentrated H_2SO_2.

The pH of this medium will be about 8.8 and may be adjusted to any desired pH below 8.8 to 7.0 with 1 M HCl: a pH of 8.1 - 8.2 was used in all experiments.

Fig. 2.1. Cell concentrations of Ankistrodesmus sp. and Selenastrum capricornutum cultured in TBIM, as a function of time, determined via turbidity (A) and by means of direct counting in an haemacytometer (B).


A
Turbidity
100
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
B
Number of cells
1000
600
400
200
100
60
40
20
10
6
4
2
Selenastrum capricornutum
Ankistrodesmus sp.
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
Elapsed time in days

Rexdale, Ontario (Plates 2.1, 2.2). An inoculum from the stock culture gave initial populations of 6.5×10^4 and 96.5×10^4 cells/ml for Ankistrodesmus and Selenastrum respectively. Cell concentrations, and thus total culture mass, were measured directly by counting in a haemacytometer and independently from turbidity (Spectronic 20, Bausch and Lomb). Relative growth rates determined by both methods are depicted in Fig. 2.1. There is no apparent reason for the transient drop in cell numbers of Selenastrum over the 13 to 14 day growth period. The growth medium used was Tris-buffered inorganic (TBIM) made up according to the recipe given by Smith and Wiedeman (1964), except for the addition of $Na_2Mo_4.2H_2O$ at a concentration of 1.19 g/l. The composition of the growth medium is given in Table 2.2.

The algae were grown aseptically in batch cultures of 100 ml in 250 ml Erlenmeyer flasks at a temperature of 13-16°C. Illumination was provided on a 14/10 hour light/dark cycle by fluorescent lamps.

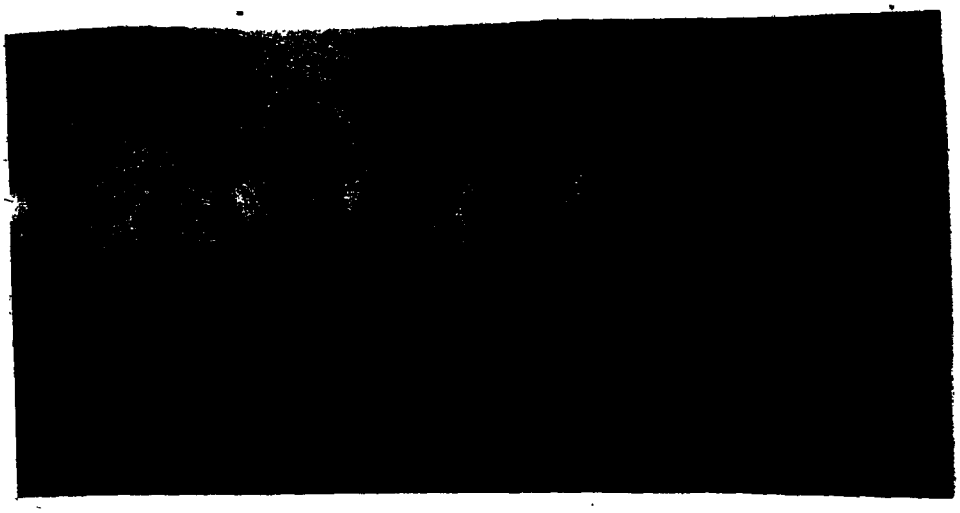
All culture flasks and glassware were prewashed in 50% HCl to minimize trace metal contamination.

Elements used for evaluation of algal uptake were Ba, Co, Ni, V, and U made up from $BaCl_2.2H_2O$, $CoSO_4.7H_2O$, $NiSO_4.7H_2O$, $VOSO_4.5H_2O$, and, $UO_2(NO_3)_2.6H_2O$ all in deionized water (DIW).

Growth conditions prior to metal exposure, and in the presence of metals (at 2 ppm) were as follows. For

Plate 2.3 A. Cultures of Ankistrodesmus exposed to culture media spiked with 2 ppm dissolved metals. Experimental Series 1. From left to right - control, Ba, Co, Ni, V. U, all metals. Note more prolific algal growth in control.

B. Cultures of Ankistrodesmus exposed to culture media spiked with 2 ppm dissolved metals for SEM/TEM micrograph. From left to right, first four Ba 2 ppm, fifth control, right hand four U 2 ppm.



Selenastrum, 14 days growth, with 3 additional days after metal introduction (1-S(3)) and 14 days growth with 22 additional days in the presence of metals (1-S(22)). For Ankistrodesmus, (a) 20 days growth, with 3 additional days after metal introduction (1-A(3)) and, (b) 21 days growth, followed by 16 days in the presence of metals (1-A(16)). Finally, for a multimetal spike, Selenastrum and Ankistrodesmus were grown together for 16 days, then 1 ml of 200 ppm stock of each metal was introduced into the same Erlenmeyer flask for 16 days exposure to the metals (Plate 2.3), followed by analysis (1-A,S(16)).

At the termination of experiments, cells were separated from the metal-spiked culture media by vacuum filtration through a millipore RA 0.2-10 μm pore size, 47 mm diameter glass fiber filter papers, dried at 65 ± 5°C for 70 hours prior to weighing. After filtration, samples were washed with DIW dried and reweighed to determine the mass of algae. Subsequently they were digested in covered teflon beakers in a boiling mixture of nitric and perchloric acids (3 ml of each) for 3 hours. The digestates were diluted to 10 ml with DIW and analysed for the elements of interest by means of inductively coupled plasma emission spectroscopy (ICP), using appropriate blanks and internal aqueous standards of 2, 0.5 and 0.1 ppm for calibration. U was determined fluorometrically, utilising an extraction step to circumvent the suppression of U fluorescence by transition

metals (see Table 2.3; Appendix I). All analyses were conducted by Barringer Magenta Ltd. of Toronto.

2.2.2 Experimental Series - 2

In these experiments metals in solutions were dripped through an algal bed to assess uptake under rapid single pass conditions for comparison to the long duration 14 to 22 day exposure conditions of experimental series 1. Ankistrodesmus was grown under identical conditions as previously mentioned above. Six 1 litre Erlenmeyer flasks were each filled with 500 ml of TBIM and inoculated with 1 ml of 177 x 10^4 cells/ml from the stock culture. After 14 days of growth the medium in each flask was divided into half (250 ml) and the algae separated by vacuum filtration, using a Millipore filter type RA 1.2 µm. Algae were washed with 10 ml DIW three times. Subsequently, every 2 minutes, 2 ml of metal-spiked solution was pipetted onto the algal mat, giving a flow rate of 1 ml/minute, and an experimental duration of 100 mins. Spiked solutions containing the metal at 2 ppm were made up from TBIM solutions containing the metal at a level of 2 ppm; or from DIW containing the metal at 2 ppm. Controls were run with TBIM and with DIW separately.

After the 100 ml of 2 ppm metal-spiked solutions had passed through, the algae were washed with 10 ml DIW three times. Algae with filter substrates were digested with HNO_3

and $HClO_4$ according to the same procedure as described above. Filtrates were evaporated to <5 ml, acidified with 3 ml 100% HNO_3 and taken to 10 ml with DIW. Algae and filtrate solutions were analysed for the metals in question as described above (see Table 2.4).

Blanks were made up by evaporating 100 ml aliquots of DIW or TBIM in teflon beakers, and taking to volume as for the other analyates. The control solutions probably inherited some barium from the experimental glassware apparatus, the measured Ba amounting to 0.7 µg, or equivalent to <0.3% of the total Ba introduced in spiked solutions, and to <1% of the Ba adsorbed by the algae (Table 2.4).

2.2.3 Experimental Series - 3

The purpose of these experiments was to determine the uptake by Ankistrodesmus of U, Ba, Co and Ni present at much lower levels of 20 to 40 ppb in solution. Given the low metal solute concentration, 10 litre volumes of metal-spiked solutions were employed in order that abundances in the products, which had undergone concentration by evaporation, would be within the limits of analytical sensitivity. Six 1 litre Erlenmeyer flasks were each filled with 500 ml TBIM and inoculated with 1 ml of 179×10^4 cells/ml from the stock culture. After 19 days of growth the medium in each flask was divided into half (~250 ml) and the algae separat-

Fig. 2.2. Experimental series - 3. Cumulative volume of filtrate from metal spiked solutions passing through an algal mat, as a function of time. Varied rates of filtration between samples are the result of individual millipore filter characteristics: the transient perturbation in filtration rate at 6-8 days is due to shut down of the vacuum line.

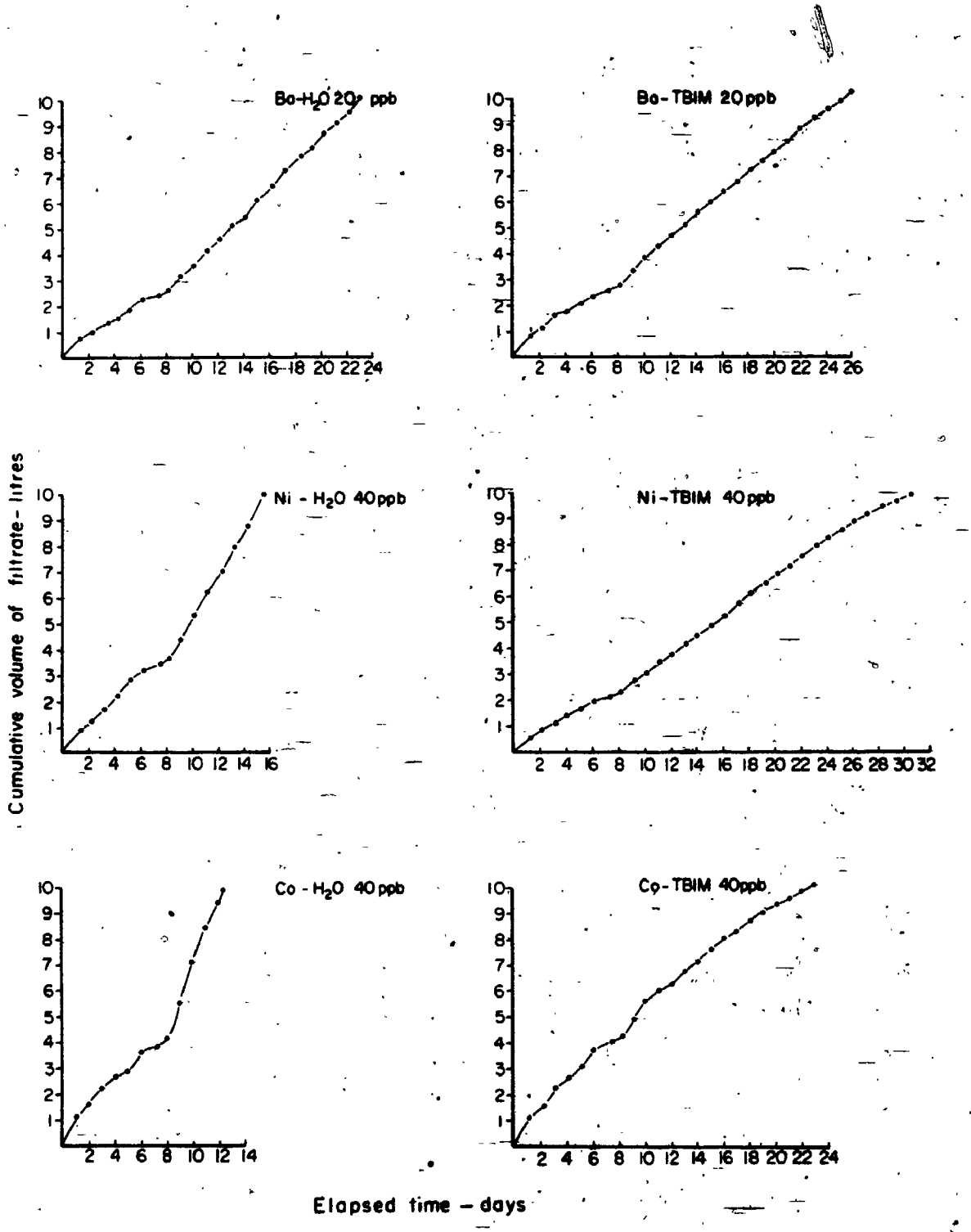
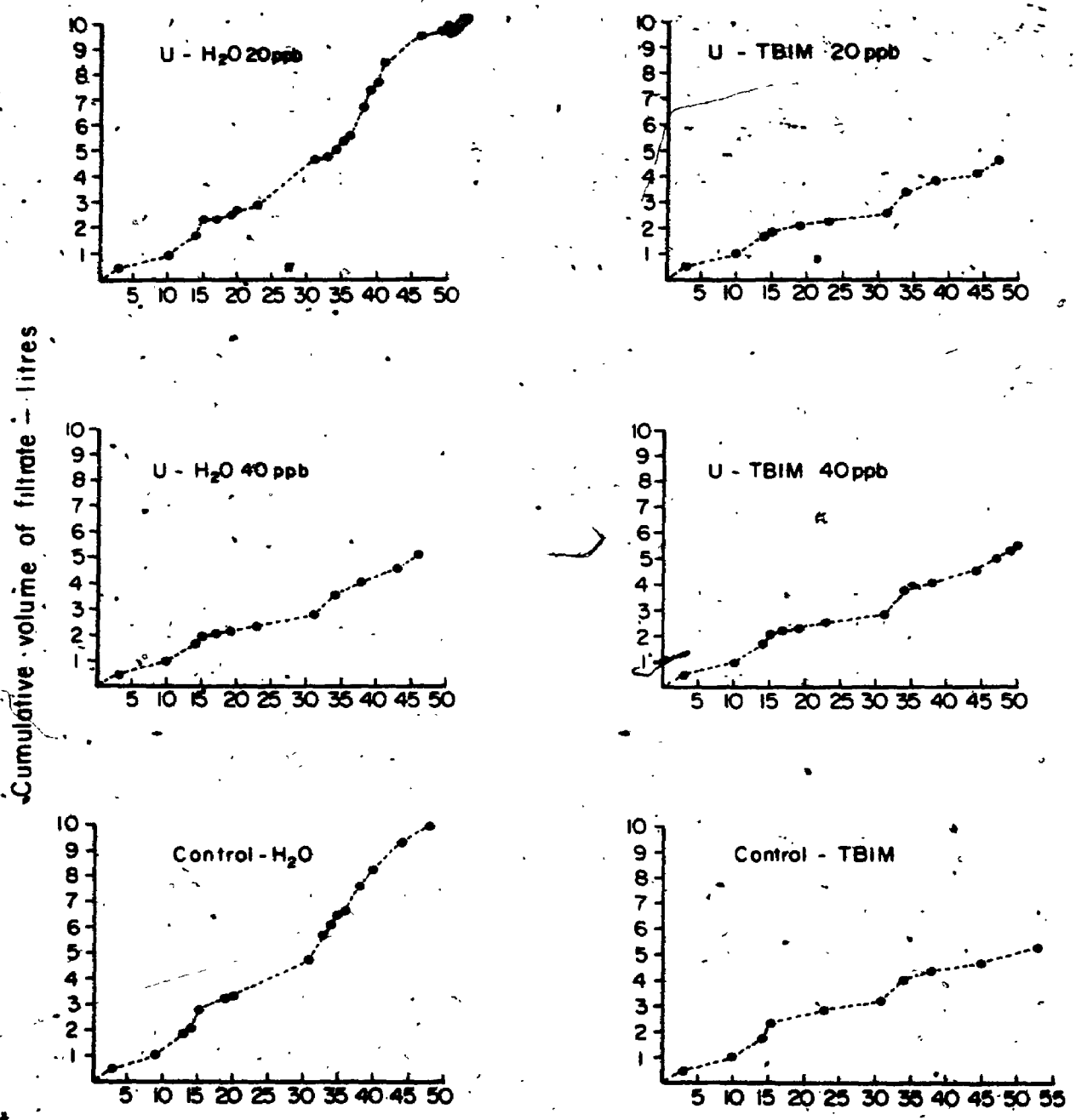

Co-H2O 20ppb
Co-TBIM 20ppb
Ni - H2O 40ppb
Ni-TBIM 40ppb
Co - H2O 40ppb
Co-TBIM 40ppb
Cumulative volume of filtrate- litres
Elapsed time – days

Fig. 2.3. Experimental series - 3. Cumulative volume of filtrate from metal spiked solutions passing through an algal mat, as a function of time.


U - H_2O 20ppb
U - TBIM 20 ppb
U - H_2O 40 ppb
U - TBIM 40ppb
Control - H_2O
Control - TBIM
Cumulative volume of filtrate — litres
Elapsed time — days

ed by vacuum filtration. The algae were washed three times with 10 ml DIW.

Metal-spiked solutions passed through the algae were made up in 10 litre volumes containing 98 ml of TBIM and were metal-spiked to give levels of 20 ppb for Ba, and 40 ppb for Co and Ni, and 20 ppb and 40 ppb for uranium. Algae were analyzed by the procedure indicated above and results are given in Tables 2.5-7. The 10 litre volumes of metal-spiked solutions were passed through the algal mats by means of vacuum filtration, requiring durations of 14 to 32 days for passage of the entire solution volumes (Figs. 2.2, 2.3). To evaluate possible differences between having TBIM present in solution versus DIW alone, metal-spiked solutions were made up as above, but omitting the addition of TBIM.

Two 10 litre controls were run through algal mats, one containing TBIM in the same proportion as for the experiments, and one with DIW alone. These blanks showed no significant U or Ba present (Table 2.6). Filtrates were evaporated to <10 ml, acidified with 3 ml concentrated HNO_3, taken to 10 ml volumes, and analysed for U and Ba.

For experiments which involve detecting transfer of nanogram quantities of a metal from one reservoir (the solution) to another (the biomass) questions of contamination, or loss by absorption to the apparatus, are of serious concern.

Given the large scale apparatus in experimental series

3, plus a long filtration duration, the glassware and filtrate receptacle were separately washed with 50% HNO_3 at termination of the experiment, the washings preconcentrated by evaporation and analysed accordingly. These metal budgets are reported in Tables 2.5 and 2.6.

In the case of uranium, that present in algae, filtrate and apparatus summed on average to within 7% of the total U originally introduced. Uranium adsorbed onto the apparatus accounted on average for 7% of the metal available for algal uptake. For barium, the separate analyates summed on average to within 95% of the total Ba introduced, and the apparatus claimed a mean of 6% of the Ba available. Results for experimental series 3 are summarized in Table 2.7.

2.2.4 Experimental Series - 4

Given the small absolute quantities of metals present in the previous experiments (200 µg, for experimental series 3), especially in algae exposed to Co, Ni, V, these experiments were conducted in an apparatus that was a factor of five larger than before in order to increase culture volumes, algal mass and absolute metal quantities, at given metal solute concentrations. The primary objective was to compare metal uptake under conditions where culture media were spiked at the outset, as against introduction of metals after an initial growth period of several days. In part A (4i) Co, Ni and V were present at 2 ppm, whereas U and Ba

were at 200 ppb.

Twelve 1 litre Erlenmeyer flasks were each filled with 500 ml TBIM, and inoculated with Ankistrodesmus sp. from a culture in which the cell concentration was 162 x 10^4 cells ml^{-1}. In part A, 5 ml of 200 ppm stock solutions of Co, Ni and V, were individually pipetted into a flask, giving 2 ppm Co, Ni, V and 200 ppb, Ba and U; one flask served as a control. For part B, metals were introduced at the same concentrations but after 16 days growth.

Algae were harvested after 17 days exposure to the spiked media in both parts A and B, using an RA 0.45 µm Millipore filter paper, and vacuum filtration. Washing of the algae, drying, weighing, digestions and analysis of the specified metals were conducted as described above. Data are reported in Table 2.8.

Given readily detectable algal uptake of Ba and U from solutions containing 200 to 40 ppb, but not of Co, Ni, or V from 2 ppm spiked culture media, the experiments were repeated (4ii), using the same levels of Ba and U to establish reproducibility of results, but at 10 ppm for Co, Ni, V to assess toxic metal levels, and further increase the absolute quantities of metals present (5000 µg). In all other respects the experiments were conducted as for 4(i), except for inoculation cell counts of 126.9 x 10^4 cell ml^{-1} in 4(ii). Results are given in Table 2.9.

2.2.5 Experimental Series - 5

These experiments were designed to establish the uptake of Ba and U on algal cell walls and membranes alone. Four Erlenmeyer flasks containing 100 ml TBIM were each inoculated with Ankistrodesmus sp. from a culture in which the cell count was 116 x 10^4 cells/ml. The Erlenmeyer flasks were shaken once a day for the 25 day duration of growth. Algal cultures from all 4 flasks were centrifuged to concentrate the algae, the excess TBIM being decanted and discarded. Centrifugation was repeated until a total of 35 ml of thick algal suspension was obtained, then 5 ml of DIW was added to bring the volume to 40 ml. In order to separate algal membranes from intracellular material cells in the concentrated suspension were ruptured by means of a chilled French Press operating at a pressure of 1200 psi (Norris and Ribbons, 1971). After a second pass microscopic inspection revealed that 95% rupture of algal cells was achieved.

The treated suspension was divided into four equal quantities of 10 ml. Each 10 ml aliquot was filtered through a type RA 0.45 µm Millipore filter paper. Filter papers became yellowish whereas the filtrate was green. If chlorophyl is an index of the cytoplasmic constituents, then this indicates that most of the intracellular protoplasm was in the filtrate, leaving cell membranes retained on the filter. Each filter paper was washed with 10 ml DIW.

One hundred ml quantities of solutions spiked with 2

ppm solute concentrations of Ba or U were passed through the membrane mats, over a duration of 100 minutes. Solutions were made up from 99 ml of DIW or TBIM and 1 ml of 200 ppm metal stock. The control consisted of 100 ml DIW. Filtrates were evaporated to <10 ml, acidified with 1 ml concentrated HNO_3 and taken to a volume of 10 ml for analysis. Filter papers were digested as described above, acidified and taken to 10 ml.

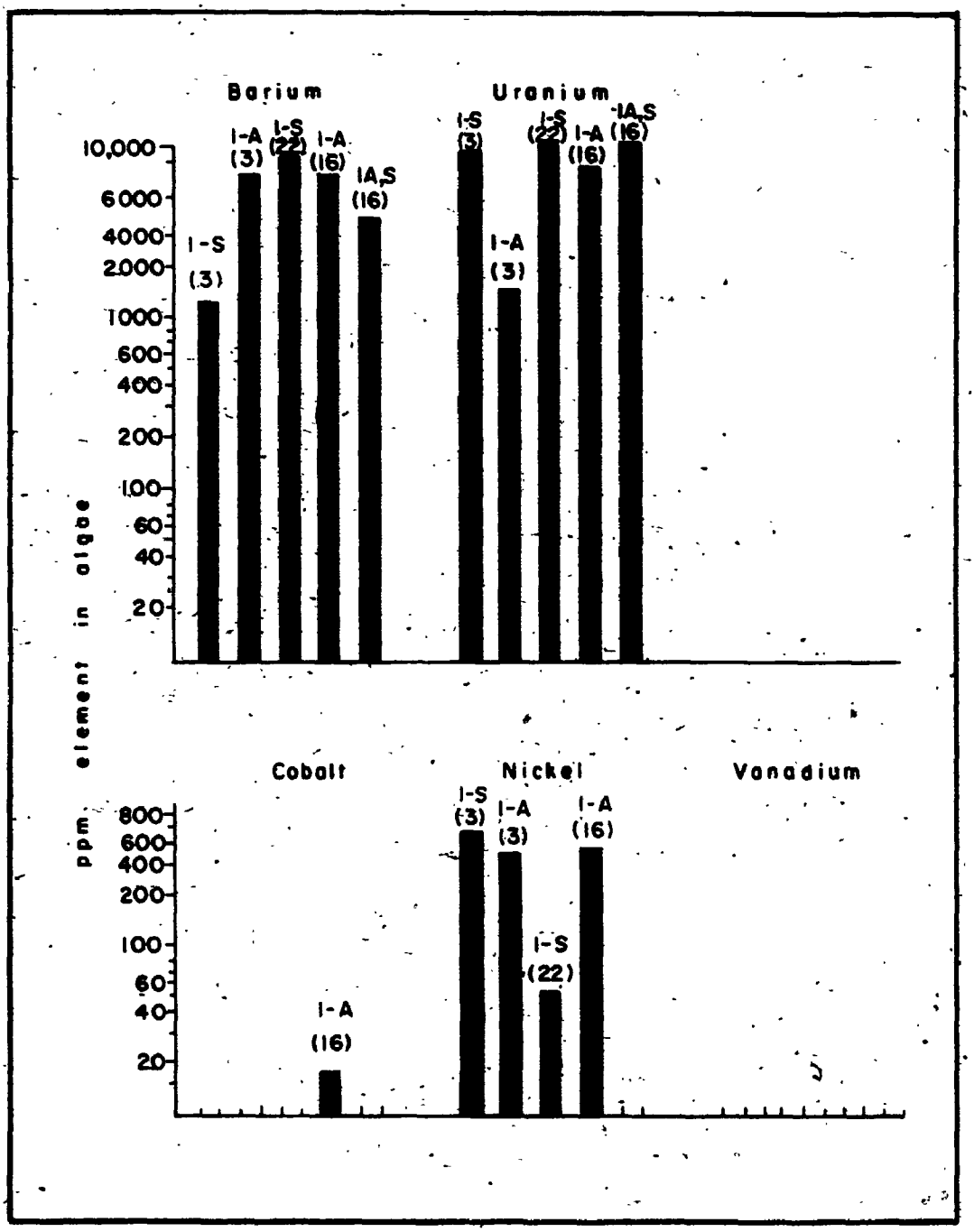
In all cases, the analytically determined g U or Ba associated with algae plus filtrate summed to within 3% of the 200 µg metal initially introduced in the metal spiked solutions (Table 2.10).

2.2.6 Experimental Series - 6

In order to examine the uptake of uranium by algae at much lower U solute concentrations of ∿1 ppb, this following experiment was conducted in a large aquarium. The aquarium was filled with 160 litres water from Fanshawe Lake, London, Ontario, on the 12th of October 1979. Over the next three years the aquarium was maintained under steady conditions, with appropriate oxygenation, circulation and 14/10 hr light/dark cycles. During this time an equilibrium population of microorganisms became established, dominated by filamentous and unicellular algae (Plate 2.4, Table 2.11).

Prior to metal introduction, a 1 litre aliquot of water

Fig. 2.4. Experimental series - 1. Concentration of metals in algae. S = Selenastrum; A = Ankistrodesmus; A,S = both algae grown in 100 ml TBIM culture medium spiked with 2 ppm (2 mg/kg) solute concentrations of the specified metals, and calculated for dry weight of algae. The figures in parentheses represent the number of days the algae were cultured in metal spiked solutions.


Barium
Uranium
Cobalt
Nickel
Vanadium
ppm element in algae
10,000
6000
4000
2000
1000
600
400
200
100
60
40
20
800
I-S (3)
I-A (3)
I-S (22)
I-A (16)
IA,S (16)

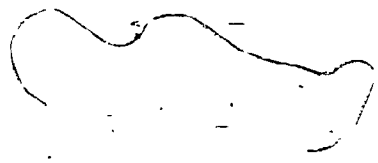
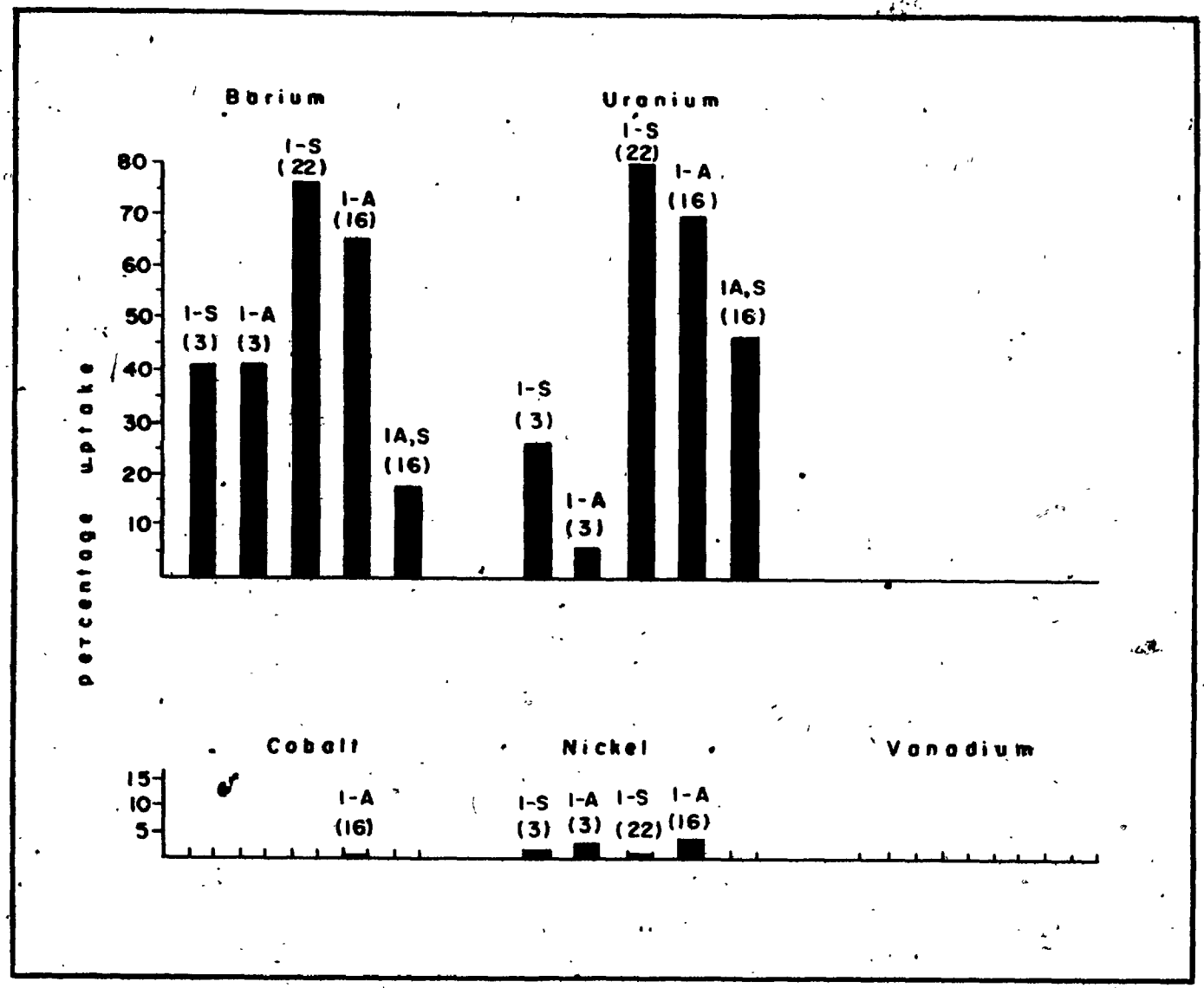


Fig. 2.5. Experimental series - 1. Percentage uptake of Ba, U, Co, Ni and V by algae growing in 100 ml TBIM culture medium spiked with 2 ppm (2 mg/kg) solute concentrations of the specified metals. S = Selenastrum; A = Ankistrodesmus; A,S = both algae. The figures in parentheses represent the number of days the algae were cultured in metal spiked solutions.


Barium
Uranium
percentage uptake
80
70
60
50
40
30
20
10
I-S (3)
I-A (3)
I-S (22)
I-A (16)
IA,S (16)
I-S (3)
I-A (3)
I-S (22)
I-A (16)
IA,S (16)
Cobalt
Nickel
Vanadium
15
10
5
I-A (16)
I-S (3)
I-A (3)
I-S (22)
I-A (16)

along with separate samples of the unicellular and filamentous algae were extracted in order to determine background levels of uranium. A one litre volume of DIW containing 100 ppb U was then evenly distributed in the aquarium, raising the aqueous U concentration by 0.62 ppb. One litre aliquots of water were taken after 5 hr, 10 hr, 1, 2, 3 and 4 days. At termination of the 5 day experiment separate samples of unicellular and filamentous algae were obtained. Waters were acidified, preconcentrated and analysed as described above. Algae were drained of water, dried, weighed and digested as for previous samples.

2.3 Results

2.3.1 Experimental Series - 1

Data for metal abundances in the algae are given in Table 2.3 (S = Selenastrum, A = Ankistrodesmus, A,S =Selenastrum and Ankistrodesmus), along with algal mass. Also tabulated are metal concentrations, expressed as ppm in dry algae; percentage uptake, defined as (µg element associated with algae/total µg introduced) x 100; and concentration factor, defined as final element abundance in algae/initial solute concentration in culture medium (both in ppm). These two parameters are graphed in Figs. 2.4 and 2.5 respectively. From the data it is evident that massive uptake of U and Ba occurs for both species of algae, which accumulate these elements at levels of 1,000 to 10,000 ppm

Table 2.3. Summary of results for experimental series 1: Selenastrum and Ankistrodesmus cultures in solutions spiked with metals at a concentration of 2 ppm.

Algae	Metal	Algal mass (mg)	Metal abundance in algae (ppm)	% uptake of metals by algae	Concentration factor†
1-S (3)*	Barium	6.8	1220	41.4	6090
1-A (3)		12.3	6760	41.5	3380
1-S (22)		17.9	8580	76.7	42870
1-A (16)		19.8	6650	65.8	3330
1A,S (16)	Ba, Co, Ni, V, U	10.1	3670	18.5	1840
1-S	Cobalt	8.4	<59	<0.25	-
1-A		11.4	"	"	-
1-S		21.3	"	"	-
1-A		16.3	17.0	0.27	8.5
1-A,S	Ba, Co, Ni, V, U	10.1	<59	<0.25	-
1-S	Nickel	7.5	450	1.69	226
1-A		10.8	330	1.79	166
1-S		21.4	55	0.58	27
1-A		20.8	360	3.80	182
1-A,S	Ba, Co, Ni, V, U	10.1	<5	<0.05	-
1-S	Vanadium	8.0	<37	<0.15	-
1-A		12.1	"	"	-
1-S		21.7	"	"	-
1-A		18.8	"	"	-
1-A,S	Ba, Co, Ni, V, U	10.1	"	"	-
1-S	Uranium	6.4	8570	27.4	4290
1-A		10.7	1310	7.0	650
1-S		18.0	9040	81.3	4520
1-A		21.1	6770	71.4	3390
1-A,S	Ba, Co, Ni, V, U	10.1	9500	47.9	47250

† Concentration factor is the final metal abundance in algae divided by the initial metal solute concentration (all metals present at a starting concentration of 2 ppm [2 mg/kg]).

* S-Selenastrum, A-Ankistrodesmus, S,A-Selenastrum and Ankistrodesmus. The figures in parentheses represent the number of days the algae were cultured in metal spiked solutions.

dry weight, representing a concentration factor of ~5,000 times the initial aqueous metal solute concentration. Concentrations for 3 and 22 or 21 day exposure durations are comparable, signifying that the uptake is rapid. Between 40 and 80% of the total Ba and U was sequestered from solution by the algae; algal growth was not suppressed by the presence of either 2 ppm Ba or U relative to control cultures.

Uptake of Ba and U is not significantly suppressed in the presence of 2 ppm Co, Ni, and V (1-A,S, Fig. 2.5) indicating that the latter group of metals act neither in a synergistic nor antagonistic manner; however the algal growth was depressed relative to control cultures and single-metal exposures of Ba or U, under the multi-metal exposure conditions (Plate 2.3).

For Co, Ni and V spiked culture media, a noticeable algal uptake was registered only for Ni (40-500 ppm) representing $\leq$ 5% of the initial Ni present. Metals combined with TBIM in culture are less toxic than with no TBIM present - e.g. 2 ppm Ni (no TBIM) is toxic. Levels of Co and V in algal analyates were at or below analytical sensitivity.

2.3.2 Experimental Series - 2

Data for element abundances in the algae are given in Table 2.4, along with algal mass. As for the previous

Table 2.4. Summary of results for experimental series 2: *Ankistrodesmus* cultures in solutions of DIW or TBIM spiked with specified metals at 2 ppm concentration.

Solution	Metal/Control	Algal mass (mg)	Metal abundance in algae (ppm)	% uptake of metals by algae	Concentration factor†
2-DIW	Barium	7.8	6920	27	3460
2-TBIM		9.5	11750	56	5880
2-DIW	Cobalt	9.4	-	-	-
2-TBIM		10.8	-	-	-
2-DIW	Nickel	9.2	-	-	-
2-TBIM		10.4	176	0.91	88
2-DIW	Vanadium	8.7	12	0.05	6
2-TBIM		9.5	6	0.03	3
2-DIW	Uranium	8.7	9720	41	4710
2-TBIM		7.7	25920	99.8	12960
	CONTROLS				
2-DIW	Barium	9.6	93	-	46
2-TBIM		10.8	82	-	41
2-DIW	Cobalt	9.6	<52	-	-
2-TBIM		10.8	<46	-	-
2-DIW	Nickel	9.6	<52	-	-
2-TBIM		10.8	-	-	-
2-DIW	Vanadium	9.6	-	-	-
2-TBIM		10.8	-	-	-
2-DIW	Uranium	9.6	-	-	-
2-TBIM		10.8	-	-	-

† Concentration factor is the final metal abundance in algae divided by the initial metal solute concentration (all metals present at a starting concentration of 2 ppm [2 mg/kg]).

Fig. 2.6. Experimental series - 2. Concentration of metals in algae (Ankistrodesmus) when 100 ml TBIM culture medium, or DIW, spiked with 2 ppm (2 mg/kg) solute concentrates of Ba, U and Ni were passed through an algal mat in about 100 minutes.

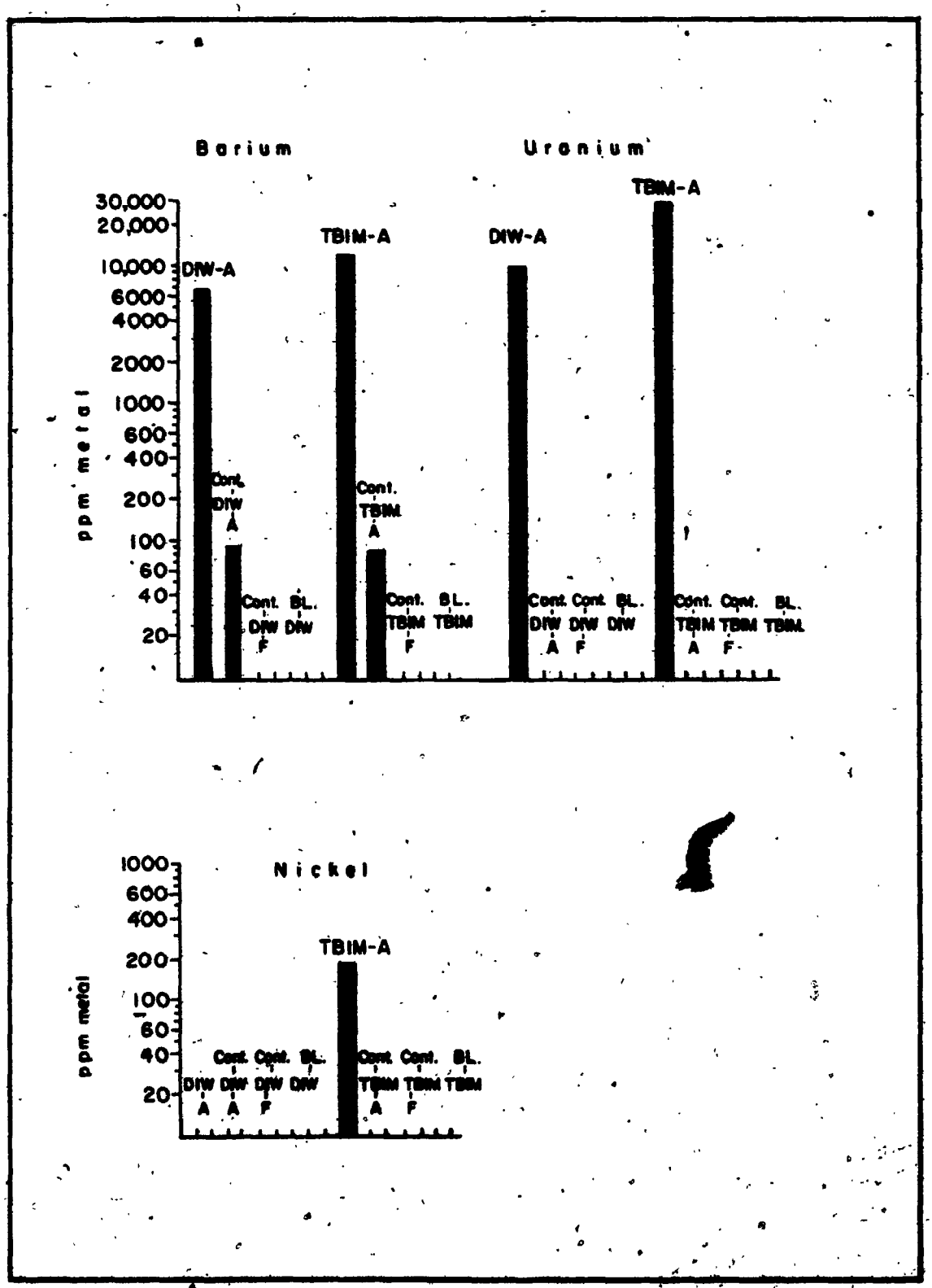
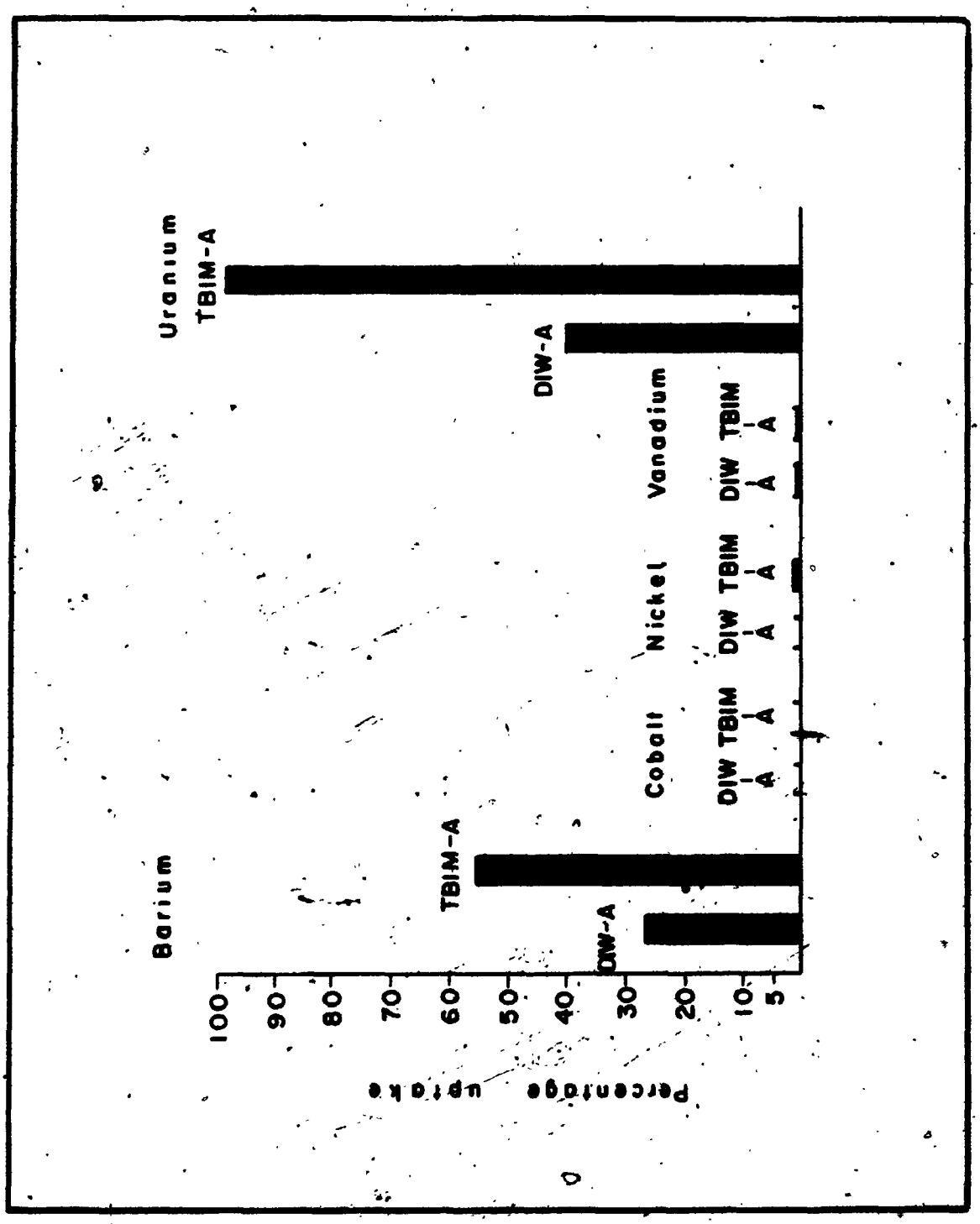

Barium
Uranium
ppm metal
30,000
20,000
10,000
6000
4000
2000
1000
600
400
200
100
60
40
20
DIW-A
Cont. DIW A
Cont. DIW F
BL. DIW
TBIM-A
Cont. TBIM A
Cont. TBIM F
BL. TBIM
DIW-A
Cont. DIW A
Cont. DIW F
BL. DIW
TBIM-A
Cont. TBIM A
Cont. TBIM F
BL. TBIM
Nickel
ppm metal
1000
600
400
200
100
60
40
20
Cont. DIW A
Cont. DIW A
Cont. DIW F
BL. DIW
TBIM-A
Cont. TBIM A
Cont. TBIM F
BL. TBIM

Fig. 2.7. Experimental series - 2. Percentage uptake of Ba, Co, Ni, V and U by Ankistrodesmus from 100 ml of solutions spiked with 2 ppm of the specified metals passing through an algal mat in about 100 minutes.


Percentage uptake
100
90
80
70
60
50
40
30
20
10
5
Barium
DIW-A
TBIM-A
Cobalt
DIW-A
TBIM-A
Nickel
DIW-A
TBIM-A
Vanadium
DIW-A
TBIM-A
Uranium
DIW-A
TBIM-A

experiment, massive uptake of Ba and U by the algae was recorded, giving 7,000 to 12,000 ppm Ba (2-DIW, 2-TBIM) and 10,000 to 26,000 ppm U (2-DIW, 2-TBIM) by dry weight. For both elements, a significantly higher uptake (2-3x) occurred when the metal was introduced in TBIM as against deionised water (Figs. 2.6, 2.7).

Despite the short duration of the experiment (~100 mins.) the percentage metal sequestered by the algal mat at 25-55% of the total for Ba (2-DIW, 2-TBIM) and 41 to 99% for U was only slightly lower (Fig. 2.7) than that recorded for the first experiment (14-22 days). This signifies that partitioning of metals from the liquid to the algae proceeds rapidly, and may implicate the cell wall as the metal receptor, given the observed rates of algal reproduction (Fig. 2.1).

Nickel was incorporated into the algae at 180 ppm, a level in the same range as obtained for the long term growth experiments. No uptake of V or Co was observed within the limits of analytical sensitivity (Fig. 2.6).

Barium was detected in control algae subject to both DIW and TBIM, at 80-90 ppm; but was not noted in either DIW or TBIM blanks. Uranium in algal controls, and U and Ba in DIW and TBIM blanks was less than analytical sensitivity (<0.1 µg Ba, 0.2 µg U). Blanks were made up by evaporating 100 ml aliquots of DIW or TBIM in teflon beakers, and taking to volume as for the other analyates. The control solutions

probably inherited Ba from the experimental glassware apparatus, the measured Ba amounting to 0.8 μg, or equivalent to <0.3% of the total Ba introduced, and to <1% of the Ba adsorbed by algae.

2.3.3 Experimental Series - 3

Results for algal uptake of metals from solutions containing 20 and 40 ppb solute concentrations respectively are reported in Tables 2.5-2.7. Levels of cobalt and nickel in algae were at or below analytical sensitivity, such that the extent of uptake of these metals could not be determined at levels approaching those of natural freshwaters with the existing experimental apparatus and analytical method.

The algae sequestered barium to 3,390 and 2,880 ppm by dry weight from solutions containing 20 ppb Ba, the higher value with TBIM present. These figures correspond to 23 (TBIM) and 17.3 (DIW only) percent uptake of the total barium introduced, and translate into concentration factors of 170,000 and 144,000 times the initial Ba solute abundances respectively (Fig. 2.8).

Uranium was incorporated by algae at levels of 3,000 to 4,000 ppm, and 6,500 ppm from solutions containing 20 and 40 ppb U respectively. In each case the presence of TBIM appeared to make little difference to uptake levels. The measured algal uranium abundances correspond to 170,000 to 200,000 (20 ppb spiked solutions) to 152,000-160,000 (40

Table 2.5. Details of U pathways for experimental series 3.

20 ppb U in TBIM-DIW	U in analyate ppm	Volume of analyate	U in analyate ug	Volume of solutions l	U present ug
Algae	8.0	10	80		80
Filtrate	0.196	100	19.6	4.65	19.6
Spiked solution	1.92	10	19.2	5.35	103
Filtrate receptacle	0.060	10	0.6		0.6
Reservoir glassware	0.970	10	9.7		9.7
Total U (ug)					213
Available U (ug)					100
20 ppb U in DIW					
Algae	3.13	10	31.3		31.3
Filtrate	1.46	100	146	9.85	146
Spiked solution					
Filtrate receptacle	1.11	10	11.1		11.1
Reservoir glassware	0.70	10	7.0		7.0
Total U (ug)					195
Available U (ug)					188
40 ppb U in TBIM-DIW					
Algae	10.6	10	106		106
Filtrate	1.54	100	154	4.45	154
Spiked solution	0.240	10	24.0	5.20(11)	125
Filtrate receptacle	0.047	10	4.7		0.47
Reservoir glassware	0.55	10	5.5		5.5
Total U (ug)					391
Available U (ug)					260
40 ppb U in DIW					
Algae	10.9	10	109		109
Filtrate	1.65	100	165	5.10	165
Spiked solution	2.59	10	25.9	4.90(11)	127
Filtrate receptacle	0.93	10	9.3		9.3
Reservoir glassware	0.76	10	7.6		7.6
Total (ug)					418
Available U (ug)					283

Figures in parentheses refer to the aliquot volume extracted from the residual spiked solution for concentration by evoration into the quoted volume of analyate.

Table 2.6. Details of Ba pathways for experimental series 3.

20 ppb Ba in TBIM-DIW	Ba in analyate ppm	Volume of analyate ml	Ba in analyate µg	Volume of solutions l	Ba present µg
Algae	4.17	10	41.7		41.7
Filtrate	14.11	10	141.1	10	141.1
Spiked solution				10	
Filtrate receptacle	0.309	10	3.09		3.09
Total Ba (µg)					186
Available Ba (µg)					183
20 ppb Ba in DIW					
Algae	3.14	10	31.4		31.4
Filtrate	12.08	10	120.8	10	120.8
Spiked solution				10	
Filtrate receptacle	1.99	10	19.9		19.9
Total Ba (µg)					192
Available Ba (µg)					172
Controls					
10 l DIW-filt.	0.113	10	1.13		
10 l TBIM-DIW-filt.	0.120	10	1.20		

Table 2.7. Summary of results for experimental series 3: Ankistrodesmus cultures in solutions of DIW + TBIM or DIW along, spiked with specified metals at 20 or 40 ppb.

System	Metal	Filtration time (days)	Filtration volume (litres)	Algal mass (mg)	Metal abundance in algae (ppm)	% uptake of metals by algae	Concentration factor†
TBIM - DIW	Barium*	26	10	12.3	3,390	23	169,500
DIW		21	10	10.9	2,880	17.3	144,050
TBIM - DIW	Cobalt	21	10	13.5	-	-	-
DIW		12	10	8.0	-	-	-
TBIM - DIW	Nickel	31	10	1.1	-	-	-
DIW		15	10	7.3	-	-	-
TBIM - DIW	Uranium*	46	4.5	20.3	3,350	80	197,000
DIW		50	10.0	9.4	3,330	17	166,000
TBIM - DIW	Uranium	50	5.5	17.5	6,080	41	152,000
DIW		47	5.0	16.3	6,440	38	161,000

Ba, U* = 20 ppb
Co, Ni, U = 40 ppb

† Concentration factor is final metal abundance in algae divided by the initial metal solute concentration.

ppb) over the initial U solute concentration (Table 2.7; Fig. 2.9).

2.3.4 Experimental Series - 4

After 16 days growth in metal spiked culture media 24 mg (Ba) and 30 mg (U) algae were harvested, compared to 29 mg for the control (4iA, Table 2.8). The results indicate no significant depression of algal growth rates in 0.2 ppm concentrations of these metals. However this was not the case for culture media containing Co, Ni and V at 2 ppm, where the harvested algal mass was from 60% (Ni), 50% (Co) to 30% (V) of the control. In part B, where metal introduction followed an initial 16 day period of growth the algal mass for each metal-treated culture flask was within 2% of the control harvest (4iB). Possibly, initial growth was more sensitive to toxic metals than established cultures, where the large available cell surface area could act rapidly to biosorb the metals thus decreasing their aqueous concentration.

For the repeat experiments (4iiA) harvested algal weight in Ba and U spiked culture media were within 10% of the control, and within 20% of results for the first run (4iA) over an equivalent growth duration. Ten ppm concentrations of Co, Ni and V, however, almost totally inhibited algal growth in part A, and drastically diminishing growth in part B (Table 2.9).

Fig. 2.8 Summary of results for percentage uptake of barium by Ankistrodesmus (upper), and the algal concentration factor over starting aqueous barium abundances (lower), for experiments 1 through 5 (figures above horizontal arrows). D and T signify DIW and TBIM respectively. Figures above bars indicate number of days of exposure to metals. See also section 2.2 for experimental details.

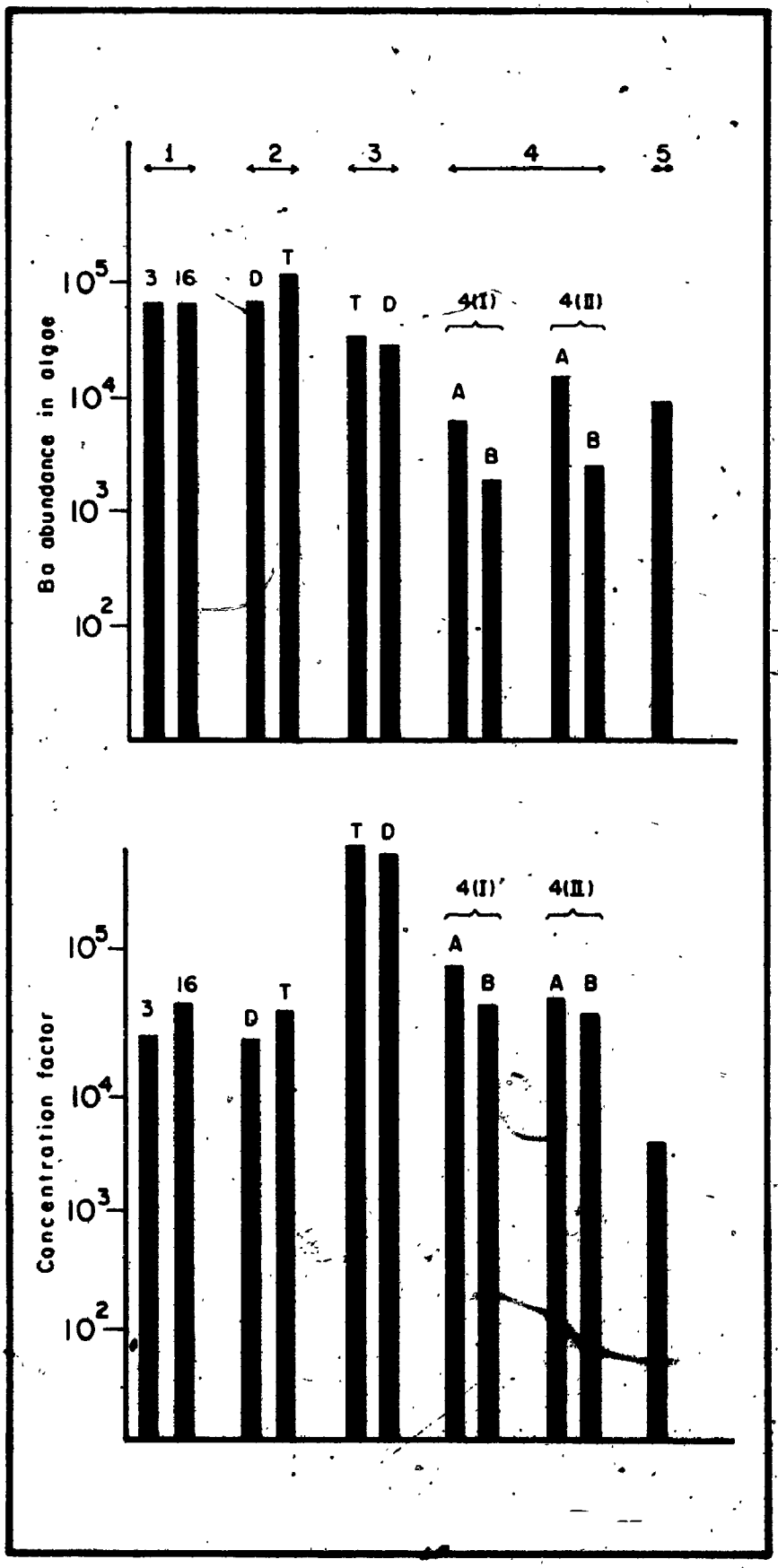

1
2
3
4
5
3
16
D
T
T
D
4(I)
4(II)
A
B
A
B
Ba abundance in algae
10^5
10^4
10^3
10^2
T
D
4(I)'
4(II)
A
B
A
B
3
16
D
T
Concentration factor
10^5
10^4
10^3
10^2

Fig. 2.9 Summary of results for percentage uptake of uranium by Ankistrodesmus (upper), and the algal concentration factor over starting aqueous uranium abundances (lower), for experiments 1 through 5 (figures above horizontal arrows). D and T signify DIW and TBIM respectively. Figures above bars indicate number of days of exposure to metals. See also section 2.2 for experimental details.

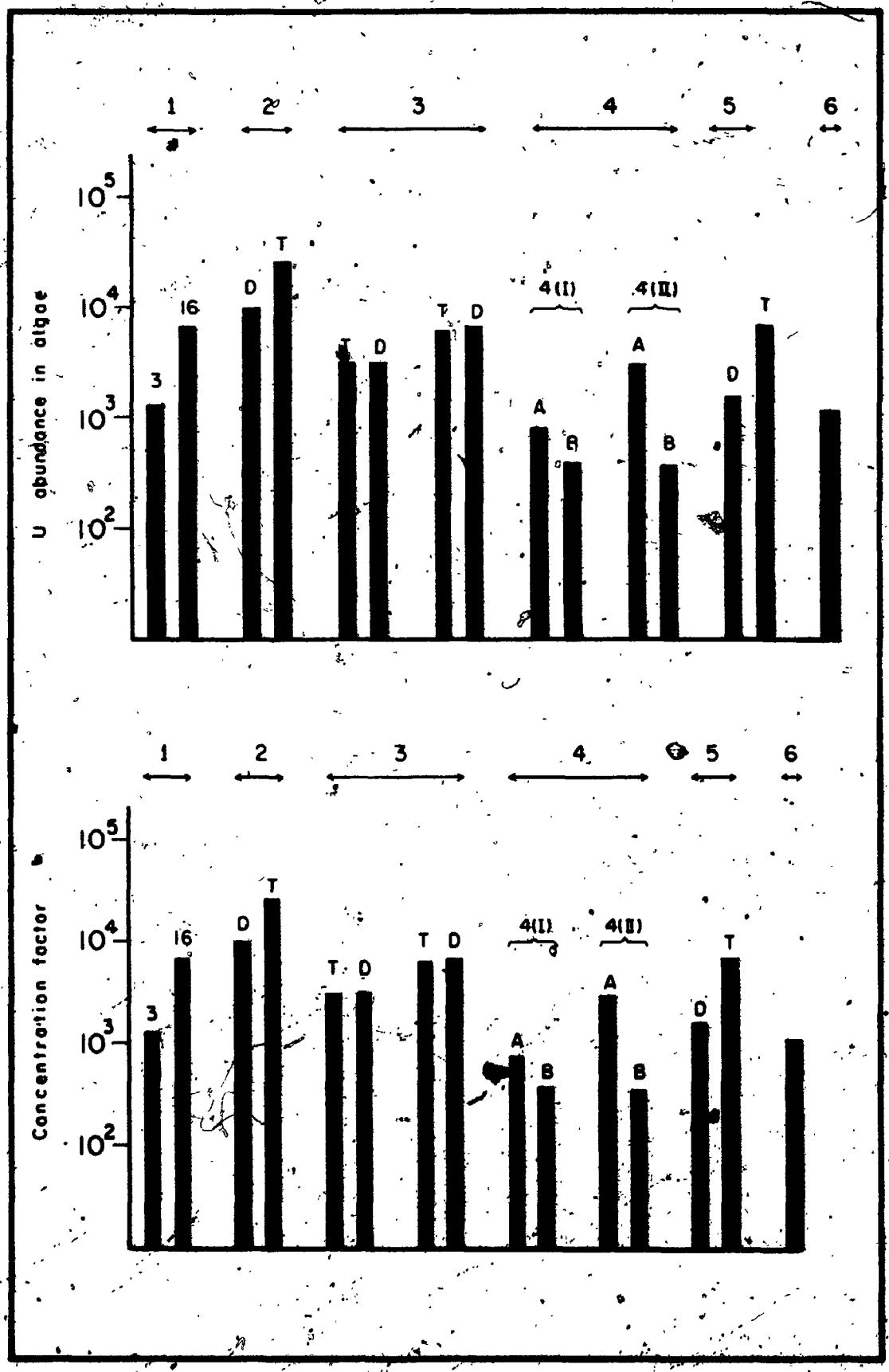

1
2
3
4
5
6
10^5
10^4
10^3
10^2
U abundance in algae
3
16
D
T
T
D
T
D
4(I)
A
B
4(II)
A
B
D
T
Concentration factor

Table 2.8. Summary of results for experimental series 4(1): Anacystis nidulans cultures in solutions spiked with specified concentrations of Ba, U, Co, Ni, V, (ppm).

Metal	Starting solute concentration	Algal mass mg	Metal abundance in algae/filtrates ppm	% uptake of metals by algae	Concentration factor
A	- 17 days exposure to metals: culture medium spiked at time of inoculation				
Ba	0.2	23.6	1,550	37	7,000
U	0.2	29.8	3,200	96	16,000
Co	2.0	14.6	<30	-	-
Ni	2.0	19.1	<28	-	-
V	2.0	11.5	45	5	22
Controls					
Ba - algae		28.6	22		
Ba - filt			0.002		
U - algae		28.6	10		
U - filt			0.0005		
Co - algae		28.6	<17		
Co - filt			0.04		
Ni - algae		28.6	<17		
Ni - filt			0.003		
V - algae		28.6	<2		
V - filt			<0.001		
B	- 17 days exposure to metals: culture medium spiked 16 days after inoculation.				
Ba	0.04	50.3	250	62	6,250
U	0.04	50.3	360	90	9,000
Co	2.0	48.8	<10		
Ni	2.0	49.2	<10		
V	2.0	50.7	10	0.06	5
Controls					
Ba - algae		48.8	6.2		
Ba - filt			0.006		
U - algae		48.7	0.24		
U - filt			6×10^{-6}		
Co - algae		48.7	<10		
Co - filt			0.06		
Ni - algae		48.7	<10		
Ni - filt			0.002		
V - algae		48.7	1.00		
V - filt			0.0001		

Metal abundances in algae from 200 ppb-spiked culture media were 1,500 ppm for Ba and 3,200 ppm for U, representing concentration factors of 7,700 (Ba) and 16,000 (U) (4iA, Table 2.8). In part B, where concentrations of 40 ppb were employed, metal abundances in the algae were much lower (280 ppm Ba, 406 ppm U). This is due both to the factor of five smaller metal quantities and the doubled algal mass, relative to part A. However, both the percentage uptake and concentration factors were comparable under the two different conditions (Figs. 2.8, 2.9). These results were reproduced in the repeat experiments - 4iiA, B (Table 2.9).

In situations where algae were exposed to 2 ppm concentrations of Co, Ni and V (4iA, B), only V was adsorbed, and then at minor levels of 10-45 ppm (Table 2.8). Repeat experiments employing 10 ppm V resulted in algal V abundances of 50-280 ppm.

Analysis of control algae and filtrates revealed Ba and U at a maximum of 2 and 0.3% respectively of metal-levels in the test algal populations. Cobalt alone was detected at an appreciable level in control filtrates, entirely in accord with its presence in the TBIM culture medium. For all other metals the filtrate controls were Ba < 1%, U < 0.2%, Ni < 0.1% and V < 0.005% of the spiking concentrations.

Table 2.9. Summary of results for experimental series 4(ii): Ankistrodesmus cultures in solutions spiked with specified concentrations of Ba, U, Co, Ni, V, (ppm).

Metal	Starting solute concentration	Algal mass mg	Metal abundance in algae/filtrates ppm	% uptake of metals by algae	Concentration factor
A	- 17 days exposure to metals, culture media spiked at time of inoculation.				
Ba	0.04	24.8	620	77	15,500
U	0.04	22.7	790	90	19,700
Co	10.0	<0.1	<5000	-	-
Ni	10.0	<0.1	<5000	-	-
V	10.0	0.6	280	0.003	28
Controls					
Ba - algae		24.3	11		
Ba - filt			0.003		
U - algae		24.3	<0.08		
U - filt			4×10^{-6}		
Co - algae		24.3	<20		
Co - filt			0.03		
Ni - algae		24.3	<20		
Ni - filt			<0.001		
V - algae		24.3	<2		
V - filt			<0.0001		
B	- 17 days exposure to metals, culture media spiked 16 days after inoculation				
Ba	0.04	49.1	280	69	7,000
U	0.04	45.3	406	92	10,200
Co	10.0	27.8	<18	-	-
Ni	10.0	28.4	170	0.10	17
V	10.0	27.8	49	0.03	5
Controls					
Ba - algae		50.3	12		
Ba - filt			0.0017		
U - algae		50.3	<0.04		
U - filt			4×10^{-6}		
Co - algae		50.3	<10		
Co - filt			0.07		
Ni - algae		50.3	<10		
Ni - filt			0.004		
V - algae		50.3	<1.0		
V - filt			0.0001		

2.3.5 Experimental Series - 5

Data for metal abundances in the algal walls and membranes are reported in Table 2.10. Uranium was taken up by the membranes at levels of 1,600 to 7,000 ppm from spiked solutions of DIW and TBIM respectively, representing concentration factors of 800 (DIW) and 3,500 (TBIM), times the initial aqueous metal concentrations. Hence transfer of U from spiked solutions to the algal walls and membranes was a factor of 4 more effective in the case of TBIM-based solutions, as compared to those made up in DIW. Between 10% (DIW) and 60% (TBIM) of the total U introduced was sequestered by the membranes, implying a high capacity for metal complexation (Fig. 2.9).

Barium was present at a final abundance of 900 ppm in the algal membranes corresponding to a concentration factor of 500 times, and 6% uptake of the total Ba introduced (Fig. 2.8).

For the controls, U and Ba were present in the algae at less than 1.8 ng and 50 ng respectively or 1×10^{-5} (U) and 2.7×10^{-4} (Ba) of the 200 µg quantities introduced for the experiments described above.

Compared to the previous experiments where live algae were employed, dead membranes were about a factor of 10 less efficient in sequestering Ba from solution, and also acquired a final metal abundance about a factor of 10 less than their live counterparts. For uranium, the membranes

were about a factor of five (DIW) and 2 (TBIM) less effective than live algae (cf. experimental series 2). In the case of both live algae and membranes alone, the effect of TBIM media was to increase the metal uptake over DIW media.

2.3.6 Experimental Series - 6

Results of experiments in which microorganisms are exposed to relatively high metal concentrations may or may not have a bearing on metal-microorganism interactions in natural systems where many metals are typically present at sub-ppb solute abundances (Holland, 1978). Hence this test, conducted in a large aquarium (Plate 2.4), extended the solute concentration of the metal spike from the 2 ppm to 20 ppb range employed in previous experiments, down to 1 ppb levels appropriate for many natural freshwaters (Bloch, 1980; Holland, 1978). An inventory of microorganisms detected in the aquarium are listed in Table 2.11.

Background levels of uranium in the aquarium water were 0.71 ppb, and 500 to 570 ppb for the algae. After introduction of the metal spike, the aqueous concentration of U rose to 1.30 ppb, ie. almost precisely by the magnitude of the 0.62 ppb artificial increment. This level diminished rapidly over one day to 0.82 ppb, and back to the starting level of 0.7 ppb at the end of 4 days (Fig. 2.10).

Samples of algae obtained at the finish had more than doubled their U contents to 1,100 ppb. Given a combined

Plate 2.4 Experimental series 6. Natural algal cultures from Fanshawe Lake propagated in an aquarium, and exposed to a 0.7 ppb uranium spike. Upper - general view of aquarium with algal growths - horizontal field of view 1.5 m. Lower - close up of filamentus algae - horizontal field of view 0.5 m.

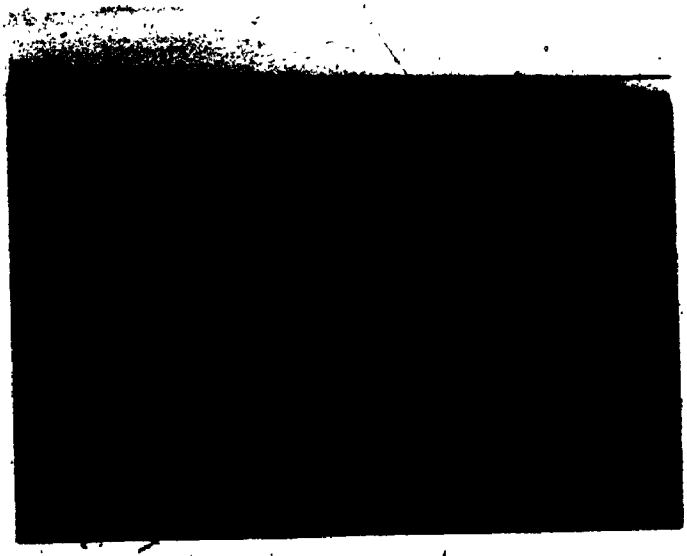


Table 2.D. Summary of results for experimental series 5: Ankistrodesmus walls and membranes exposed to solutions of DIW or TBIM spiked with specified metals at a 2 ppm concentrations.

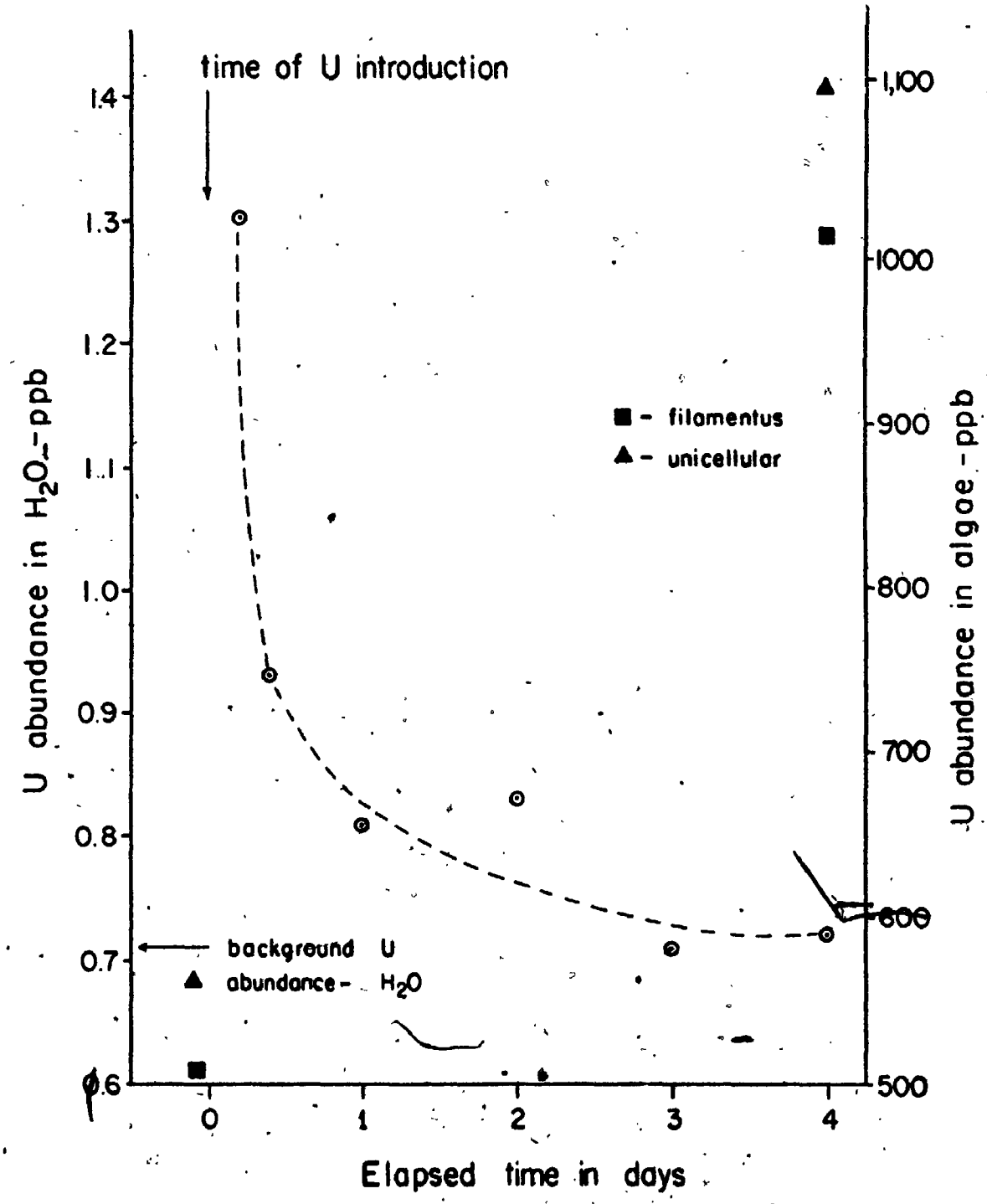
Solution	Metal	Algal Mass (mg)	Metal abundance in algae (ppm)	% uptake of metals algae	Concentration factor†
5 - DIW	Ba	13	915	5.9	460
5 - DIW	U	13	1580	10	790
5 - TBIM	U	17	6940	59	3470
5 - DIW Control	Ba	18	<3		
	U	18	<0.1		

† Concentration factor is the final metal abundance in algae divided by the initial metal solute concentration (Ba or U present at a starting concentration of 2 ppm [2 mg/kg]).

Table 2.11. List of principal algae and diatoms present in Fanshaw Lake water, and maintained in aquarium.

Algae	Diatoms
Ankistrodesmus sp.	Cyclotella sp.
Ankistrodesmus falcatus	Navicula sp.
Anabaena sp.	
Aphanizomenon flos-aquae	
Chlamydomonas globosa	
Chlorella vulgaris	
Chroococcus limneticus	
Coelastrum microporum	
Dictyosphaerium pulchellum	
Gloeocystis gigas	
Kirchneriella contorta	
Microcystis aeruginosa	
Ourococcus sp.	
Pandorina morum	
Pediastrum boryanum	
Scenedesmus sp.	
Scenedesmus dimorphus	
Scenedesmus obliquus	
Scenedesmus opoliensis	
Scenedesmus quadricauda	
Selenastrum minutum	

Fig. 2.10 Experimental series - 6. Decay of U abundance in aquarium water, following introduction of a 0.62 ppb U spike. Note starting and final U concentrations in filamentous and unicellular algae.


time of U introduction
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
U abundance in H_2O - ppb
1,100
1000
900
800
700
600
500
U abundance in algae - ppb
■ - filamentus
▲ - unicellular
background U
abundance - H_2O
0
1
2
3
4
Elapsed time in days

algal weight of 2.3 g, this represents adsorption of 1,000 ng U, or 1% of the total introduced.

It is difficult to estimate what proportion of the total aquarium biomass this 2.3 g algal sample represents, but a qualitative visual estimate is 0.5 to 2%.

In summary, uranium uptake by microorganisms from dilute solutions, rapidly restored U levels to ambient background; algae are implicated as prime candidates for the metal adsorption.

Although the incremental concentration factor of 830 (1,000-500 ppb/0.6 ppb) in this experiment is much smaller than previous tests, this may be due to the lack of available excess aqueous U. This possibility is raised by comparing the results of experimental series 3 and 4, both conducted at 40 ppb, but with the former involving 10 litre volumes (4×10^5 ng U) and the latter 0.5 litres (2×10^4 ng U). Algal concentration factors were about ten times greater in the 10 litre experiments. In a follow-up, it would be instructive to add successive 10^5 ng (0.62 ppb) increments at four day intervals, at each stage monitoring the incremental rise of algal U, and rate of decrease in aqueous U to ambient background.

The observation that U aqueous was restored rapidly to the starting value may imply that in natural freshwaters metal solute concentrations are biologically buffered.

2.4 Microscopic evidence for uranium uptake by algae

A crucial question in experimental studies of metal uptake by microorganisms is whether the metal precipitates out of the spiked solution, and is therefore not available for uptake. For several of the experiments reported here, a material balance was conducted, by comparing the sum of the metal analytically determined for the algae and spiked solution at termination, with that introduced; in all cases the mass introduced and that in the products agreed to within ± 4%, and hence the question of metal precipitation can be ruled out. This question is addressed for individual experiments in sections 2.2.3, 2.2.5, 2.3.4, and 2.3.6.

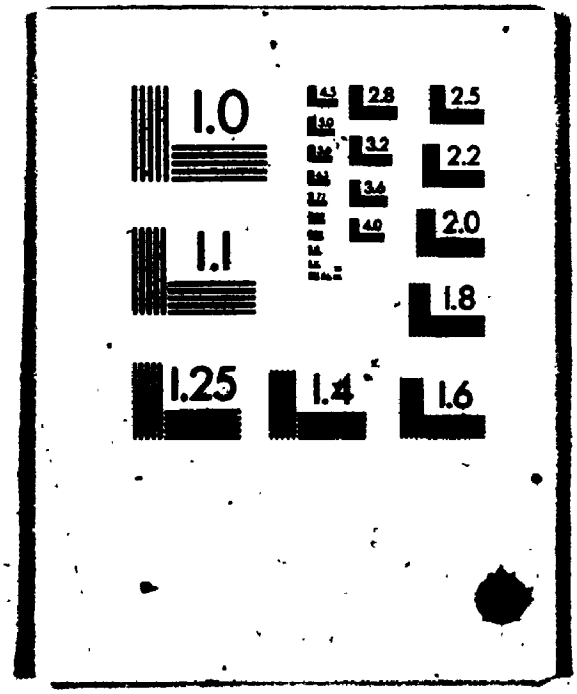
In order to establish the cellular sites of uranium deposition on Ankistrodesmus algae, a set of TEM micrographs were made of Ankistrodesmus cultured in TBIM media spiked with 2 or 5 ppm U (Plates 2.5-2.8). The micrographs were made at the University of Guelph, courtesy of Dr. T. J. Beveridge. The TEM micrographs clearly show the presence of some uranium mineral crystals, decorating the cell walls of Ankistrodesmus. The crystals have a cubic habit, and may plausibly be uraninite (UO_3) or ulrichite (UO_2) both of which are cubic and naturally occur in cubes (cf. Dana, 1932).

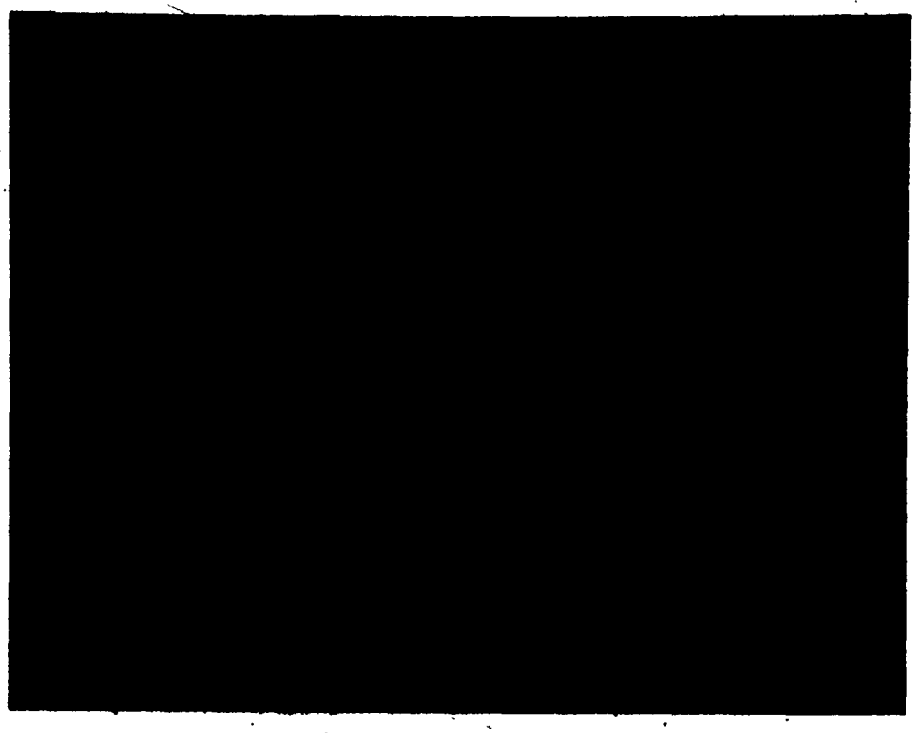
In one batch of micrographs there is only a minor amount of uranium mineral crystals on cell walls (Plate 2.7), although uranium crystals were present scattered

Plate 2.5 A. Energy dispersive spectrometer (EDS) X-ray dot map of uranium distribution around a cell of Ankistrodesmus exposed to a TBIM culture media spiked with 2 ppm U. Dot size = 20 nm (nanometers). Conditions - 60 kV, 58 μa. Magnification 17,500 diameters.

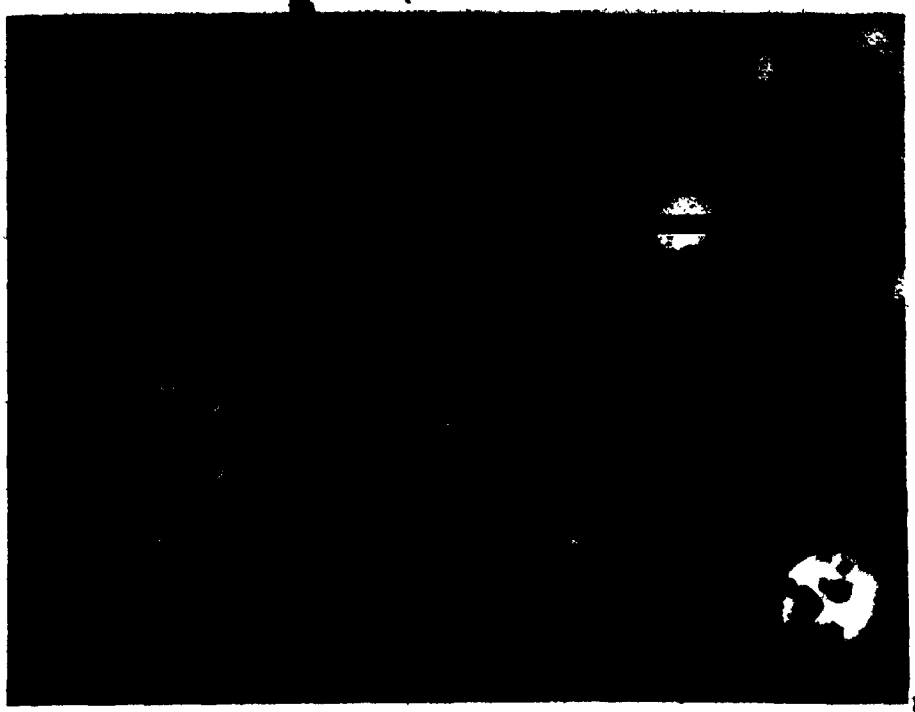
B. Bright field STEM micrograph of uranium mineral crystals decorating the cell wall of Ankistrodesmus. Same cell and treatment conditions as above. Magnification 17,000 diameters. White spot = 200 nm.

2





A



B

Plate 2.6 TEM micrograph of Ankistrodesmus sp. exposed to a TBIM culture medium spiked with 2 ppm U.

A. white spot size 40 nm, bar = 100 nm.

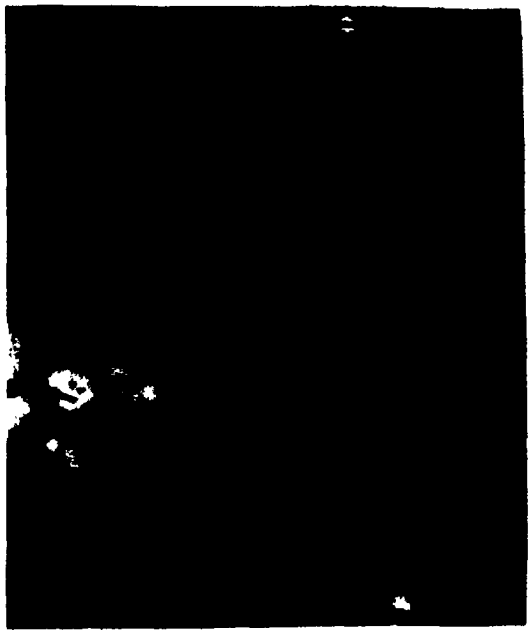
B. white spot size and bar = 100 nm.

C. white spot size and bar = 100 nm.

D. white spot size and bar = 200 nm.

A

B



C

D

Plate 2.7 TEM micrograph of Ankistrodemus sp. exposed to a TBIM culture medium spiked with 5 ppm U.

A. Ankistrodesmus with no uranium mineral on cell walls. Bar = 100 nm.

B. Ankistrodesmus with minor uraninite on cell walls. Bar = 400 nm.

C. Ankistrodesmus with no uraninite on cell walls: note deformed nature of cell walls. Uraninite crystals in upper right hand corner. Bar size = 200 nm.

D. Ankistrodesmus with no cell wall uraninite but deformed cell surfaces. Bar size = 200 nm.

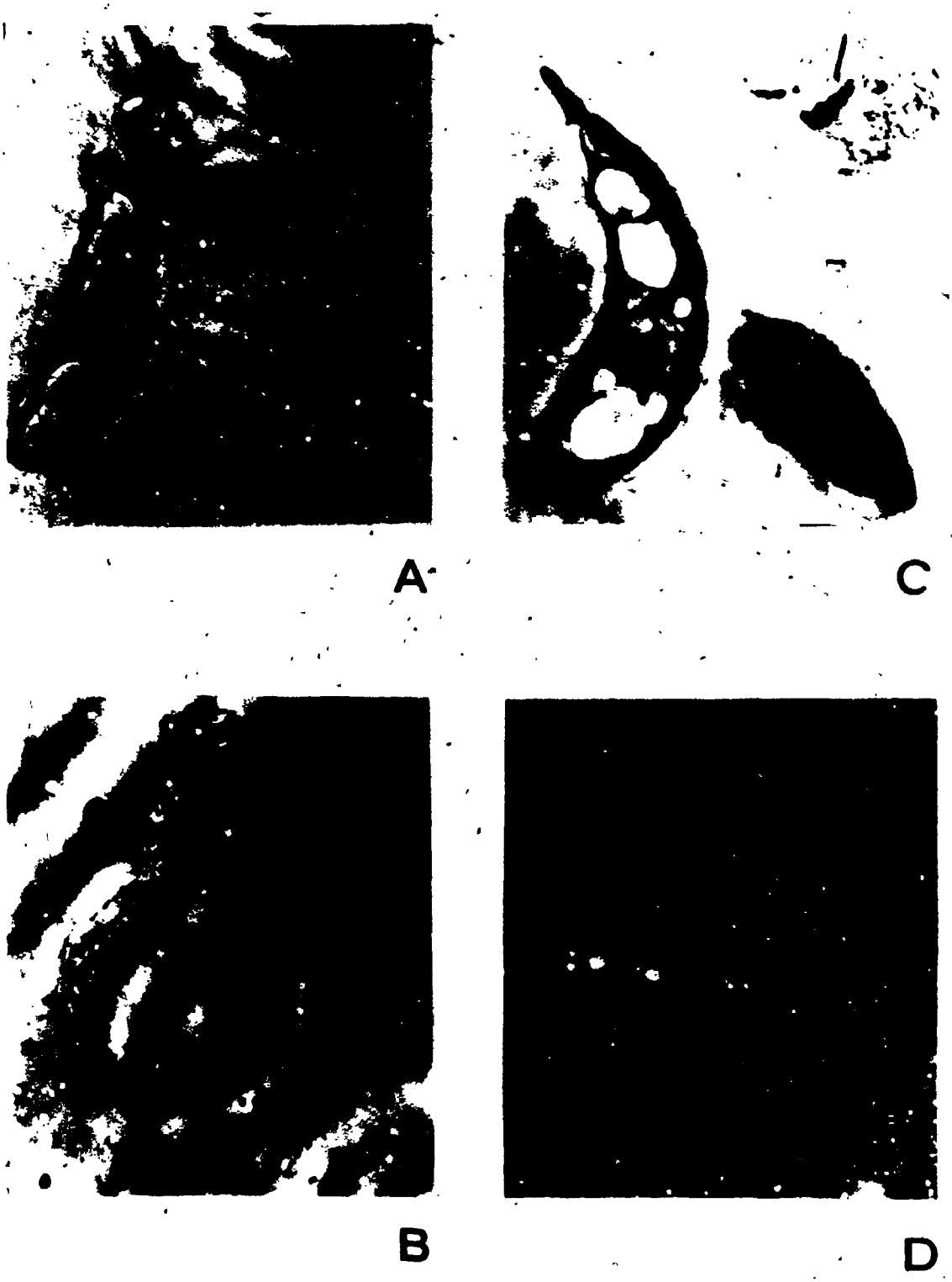
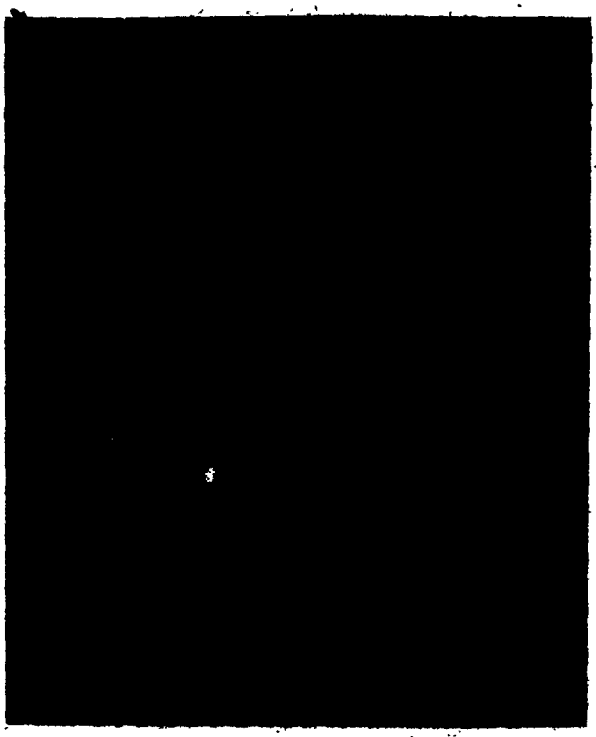

A
C
B
D

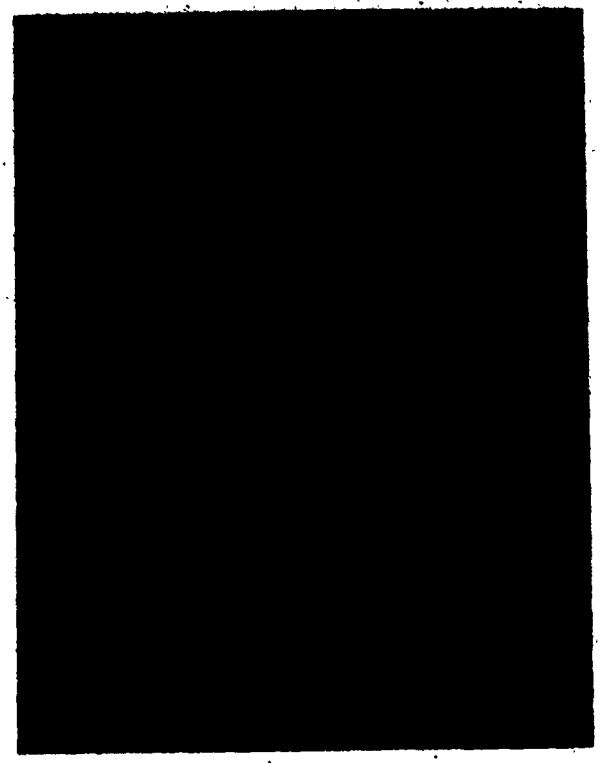
Plate 2.8 TEM micrograph of control Ankistrodesmus cultured in TBIM with no U spike.

A. Horizontal field of view 2,000 nm.

B. Bar = 200 nm.



A



B

elsewhere (Plate 2.7E, F). The reason for this is not yet apparent.

2.5 Discussion

The significance of biosorption of metals in solutions and their subsequent precipitation derives from the observation that although solute concentrations in rivers are low, the dissolved component of some metals constitutes 90% of the total metal flux, with colloids and particulates contributing only 10% (Perhac, 1972).

Experimental data for biosorption of U are available for yeasts and bacteria. Experiments for yeasts exposed to uranyl nitrate, showed rapid uptake in 2 minutes. Tuorinen and Kelly (1973) report that UO^{2+} depressed the growth of the bacterium Thiobacillus ferrooxidans at 2.5 to 4 x 10^{-5}M (6-9 ppm U), and uranyl sulphate retarded CO_2 fixation at 10^{-3} M (1200 ppm U). The inhibitory action of uranyl sulphate was reduced in the presence of 0.2 M Mg^{2+}, Mn^{2+}, Zn^{2+} and by 0.02 M EDTA. Accumulation of U by the yeast Saccharomyces cerevisiae and bacteria Pseudomonas aeruginosa up to 10-15% dry weight has been demonstrated by Strandberg et al. (1981): only 32-77% of the bacterial population appeared to possess visible cell wall U deposition. Uptake was 5-10 times the levels obtained with algae in this study (Fig. 2.9).

The fungus Rhizopus arrhizus has recently been shown to

absorb uranium from waste water, up to 18.5 percent of the dry weight of the cell. This is more than twice the uptake of commercially available ion-exchange resins (Brierley, 1982). Two principal sites for U complexation in biological systems have been proposed; membrane polyphosphates in yeast cells (Rothstein and Meier, 1951) and carboxyl and phosphoryl groups in bacteria (Beveridge and Murray, 1980; Mathews and Doyle, 1979).

In natural freshwater systems where bioconcentration of uranium is involved, such as Black Sea muds, plankton are implicated as the prime agents for U-fixation (Degens et al., 1977). The global river flux of dissolved uranium to the oceans from chemical weathering of continents is estimated at 1.92×10^{10} g/yr (Bloch, 1980). Major oceanic sinks for uranium include fixation during low temperature alteration of oceanic crust (~50%), and incorporation into organic rich marine sediments and coexisting phosphorites (~10%). However, considerations of certain continental organic-rich sediments which also possess elevated uranium abundances reveal that microorganisms may play a major role in mediating the output of uranium from continental runoff in appropriate freshwater environments. For instance Black Sea muds contain 94 ppm U (Degens et al., 1977).

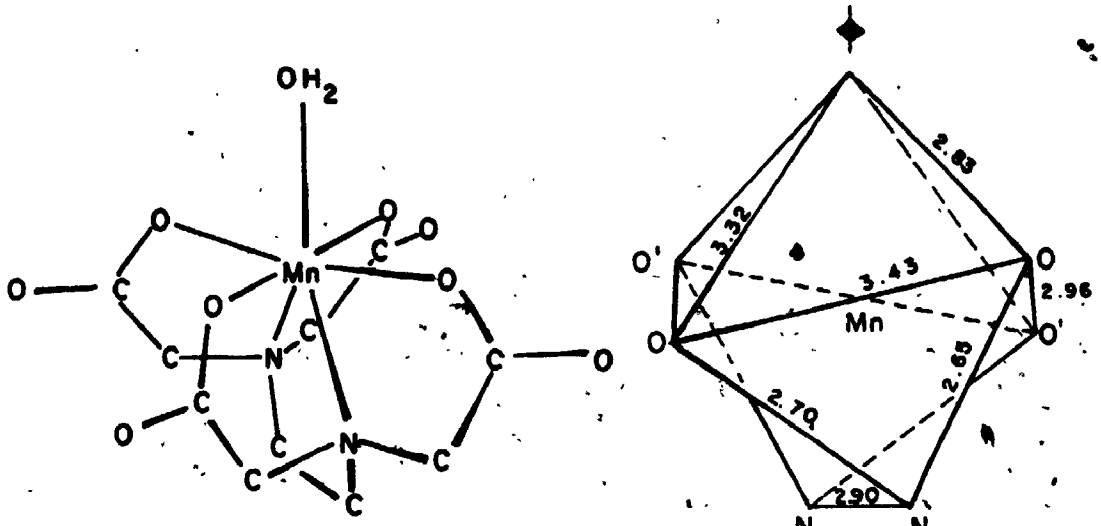
To our knowledge there is no independent experimental data for biosorption of Ba by algae. The present data show massive uptake on algae (Fig. 2.8), and if the related

alkali metal Rb behaves in natural systems as Ba does, then high bio-Rb transport from the oceans into marine sediments may have important consequences for the distribution of its radiogenic daughter ^{87}Sr. For experimental series 3, the initial barium solute concentration of 20 ppb is closely comparable to the marine abundance of 30 ppb: barium levels in the algae at 3,000 to 7,000 ppm are about one hundred times greater than that reported for 'typical' marine plants by Trudinger (1976). Beveridge (1978) reports no measurable uptake of Ba by cell walls of the bacteria Bacillus subtilis from 10 mM solutions.

Synthetic organic ligands such as ethylenediaminie-tetracetic acid (EDTA) are added to defined inorganic culture media to ensure trace elements, principally Fe and Mn, are available to support algal growth (Miller et al., 1976; Fig. 2.11). Most of the media used for routine culturing of the algae contain such chelating agents. By forming soluble complexes with the micronutrient metals, the chelating agents prevent the metals from being lost from the medium by precipitation or absorption onto the container surface (Stein, 1975, Chap. 1, p. 737), and reduce toxicity of the metals to algae (cf. Experimental Series - 2).

Chelators are very important reagants in nature. Barber and Ryther (1969) suggested that natural organic chelators, released by organisms as the water ages in the Cromwell Current upwellings, at the surface, may be partly

Fig. 2.11 Coordination complex of EDTA (ethylenediaminie-tetracetic acid) and Mn^{2+}, illustrating the site occupied by metals complexed with EDTA. Modified after Degens (1976).



HOOC-CH$_2$ N -CH$_2$-CH$_2$- N CH$_2$-COOH
HOOC-CH$_2$ CH$_2$-COOH

responsible for the increased phytoplankton growth north and south of the equator. In all studies to date, the absolute heavy metal concentration does not provide a measure of toxicity: rather the amount of chelator present is important with toxicity related to free metal concentrations (Allen et al., 1980).

Heavy metal tolerance in algae has been studied for a number of years. The present knowledge of algal uptake comes from such studies, and the use of algal bioassays to assess heavy metal toxicity of waters (Hutchinson and Stokes, 1975; Miller et al., 1976; Allen et al., 1980).

In freshwater lakes (cf. Lakes Ontario and Erie), without excessive heavy metal pollution, Cladophora glomerata concentrates Cu by 1 x 10^3 to 2.5 x 10^3 (Stokes, 1979). For Cu and Ni polluted lakes in the vicinity of Sudbury, Ontario, metal tolerant Chlorococcales grow in aqueous concentrations of 1.5 ppm Cu, and 3 ppm Ni against related strains which were inhibited at 0.1 ppm Cu and 1.0 ppm Ni (Stokes, 1975). In the experiments reported here (with TBIM as the growth medium) algal growth is not inhibited by 2 ppm Ni (Tables 2.3 and 2.4) and concentration factors were 70-600 times the aqueous concentration of 2 ppm Ni: however growth was almost totally suppressed in solutions containing 10 ppm Ni. Final nickel levels of 55 to 450 ppm in the algae (2 ppm solutions) compares to abundances of 3 ppm Ni reported by Trudinger (1976) for 'typical' marine plants

growing in ocean water at 5 ppb. The maximum recorded levels of Co (17 ppm) and V (12 ppm) in algae from 2 ppm metal spiked solutions, compares with abundances of 0.7 and 2 ppm for 'typical' marine plants growing in ocean water with 3 and 2 ppb concentrations Co and V respectively (Trudinger, 1976). Minor uptake of Co, Ni and V relative to that of U and Ba may be an algal species specific phenomenon, and/or due to more effective competition for the former group of metals by EDTA as compared to the algae (cf. Bowman et al., 1981). However, the recorded enrichments in these experiments are high relative to those reported for Co, Ni and V in 'average' marine plankton (Trudinger, 1976).

Another important role for uptake of some metals is played by pH, for example copper and nickel are less soluble, and therefore less available for uptake at higher pH's (Hutchinson and Stokes, 1975).

CHAPTER 3

THE URANIUM CONTENT OF WATERS AND AQUATIC MICRO-ORGANISMS: THAMES RIVER

3.1 Objectives and scope of study

Examination of uranium abundance in Thames River waters and selected microorganisms of its aquatic ecosystem constitutes the essential purpose of this chapter. The specific objectives were as follows:

1. To establish the concentration of dissolved uranium in Thames River waters plus some of its tributaries, and to compare these results with data for other Canadian as well as major world rivers.
2. To determine if any seasonal variations occur in dis-

solved uranium, and if so to see if such variations relate to factors such as rainfall, river flux, phosphate levels, microbiological productivity, etc.

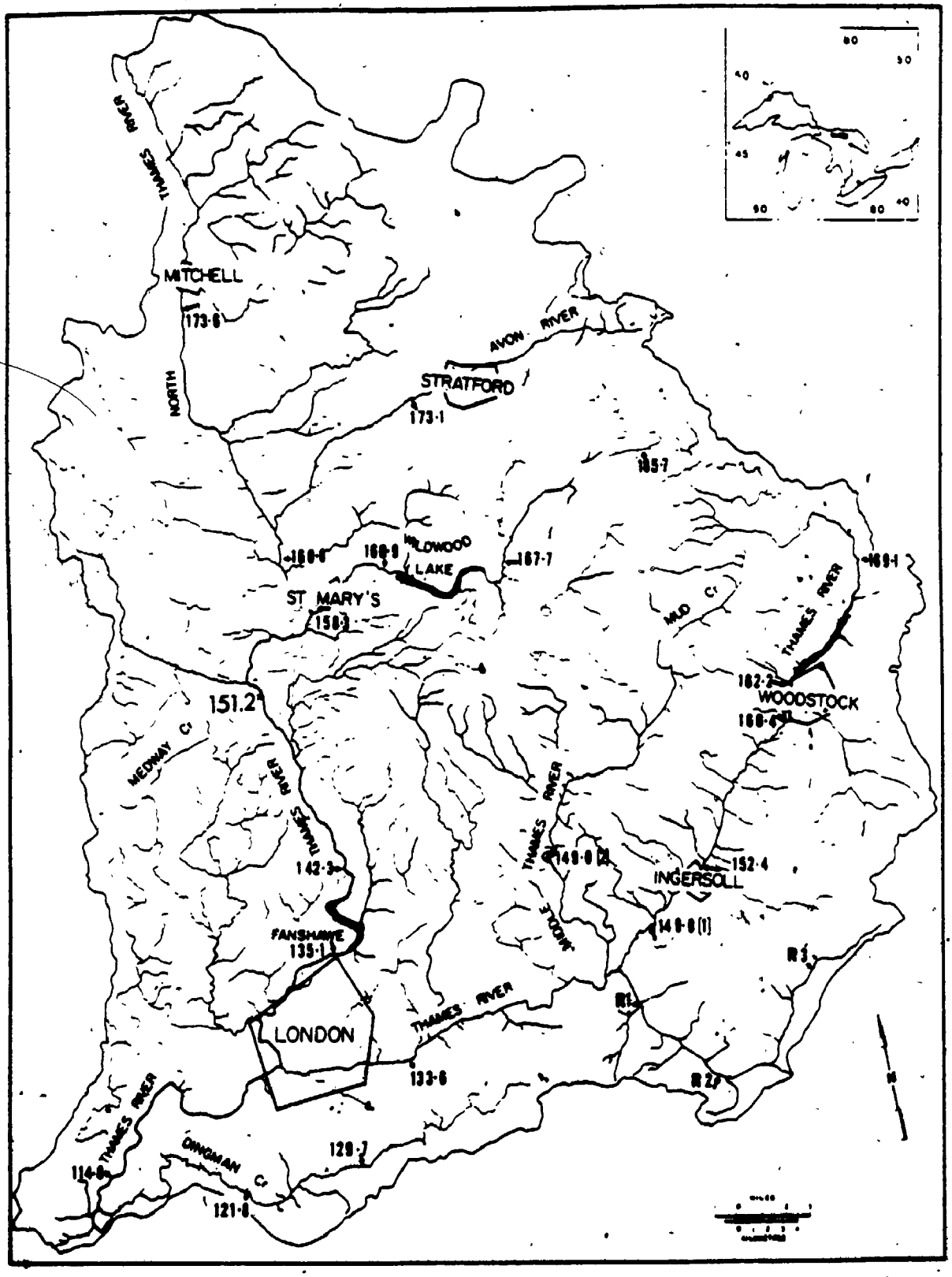
3. With reference to item two, specifically to monitor dissolved uranium during spring thawing.
4. To measure the uranium content of suspended particulates, especially algae, and colonies of filamentous algae.
5. To compare uranium levels in Thames River algae with those found in the experiments reported in chapter 2, and
6. Conduct detailed studies and chemical microanalysis of intracellular crystals found in one sample of filamentous algae.

This chapter continues with a description of the timing and details of sampling, presents in brief background information on the Thames River drainage basin, and then reports the data collected in the context of the specific objectives outlined above.

3.2 Materials and experimental methods

Two litre aliquots of Thames River waters were collected from the twenty four sampling stations illustrated in Fig. 3.1, commencing November 1981 for the twelve month interval until October 1982. For logistical reasons, and for the purpose of comparing data on the same samples,

Figure 3.1 Sampling stations for collection of water and algae, Upper Thames River and tributaries.


MITCHELL
173·6
NORTH THAMES RIVER
AVON RIVER
STRATFORD
173·1
185·7
168·6
168·8
WILDWOOD LAKE
167·7
ST MARY'S
158·3
169·1
MUD C.
THAMES RIVER
182·2
WOODSTOCK
168·6 [1]
151.2
MEDWAY C.
NITH RIVER
THAMES RIVER
142·3
149·8 [2]
152·4
INGERSOLL
MUDDY CREEK
149·8 [1]
FANSHAWE
135·1
R 3
R 1
R 2
LONDON
THAMES RIVER
133·6
129·7
THAMES RIVER
114·6
ONONDAGA C.
121·6

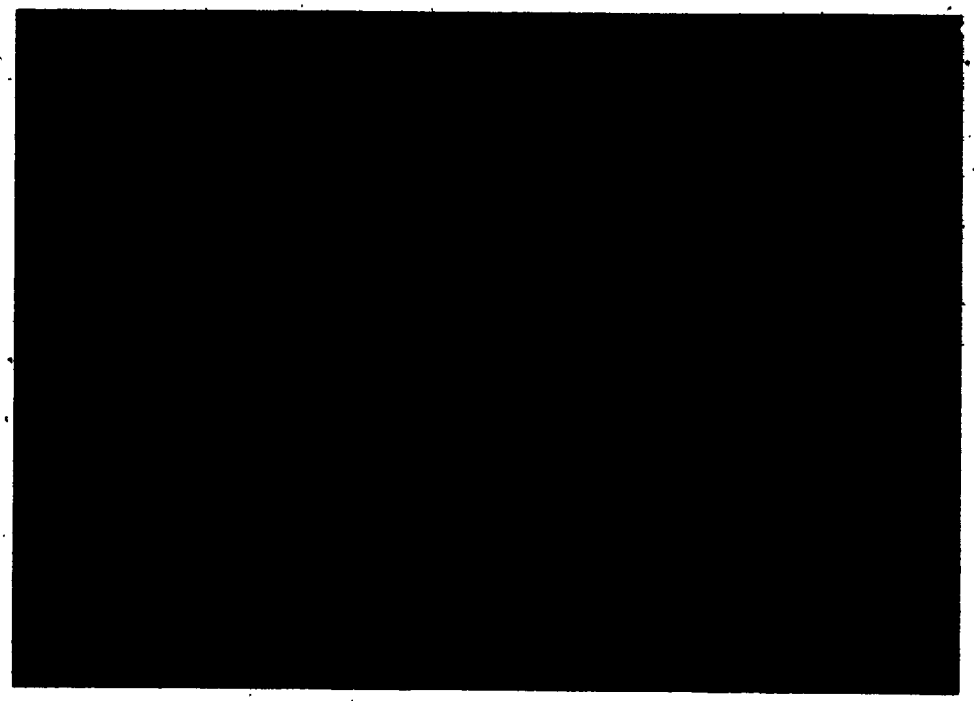
collection of waters was conducted in co-operation with the Upper Thames River Conservation Authority.

The Authority routinely monitors river parameters such as discharge rate, total and dissolved phosphate, total and dissolved solids, biological oxygen deficit, bacterial count, etc. on a monthly basis (see below). In general, sampling was performed during the third week of each calendar month; one day was destined for the northern part of the upper Thames River drainage basin, the second day being devoted to sampling the central plus lower river system.

At times when the river banks were in a safe condition, waters were collected by directly submerging two one litre nalgene bottles into the river. Each bottle was prewashed with HNO_3, then DIW; samples were acidified on site using premeasured 10 ml aliquots of reagent grade HNO_3 (see Appendix I for details). For hazardous river bank conditions, a 0.5 litre stainless steel container attached to a rope, was deployed either from a convenient bridge, or at a judicious distance from the water's edge.

In order to record the nature of suspended riverine particulates, a third one litre aliquot was collected, commencing March 1982. For the purpose of preserving algae and killing bacteria, Lugol's iodine solution was added until the water turned a medium brown colour. Lugol's solution was made up from 1 g iodine and 2 g

Plate 3.1 Upper. Thames river, filamentous algae *Draparnalida glomerata*. Location - U.W.O. grounds. Magnification 300 diameters. Lower. Diatoms (bottle shaped), and *Euglena* sp. (upper right) from the same location as above. Magnification 250 diameters.



potassium iodine in 300 ml DIW. The 1 litre volumes of water were left for 1 day, such that suspended particulates sedimented; excess water was then decanted and the residue examined both optically for microorganisms and by X-ray diffraction to determine mineral particulates.

River waters, along with suspended particulates retained on 0.45 μm filter papers, were preconcentrated by evaporation, digested and analyzed for U as described in Appendix I. In some instances samples could not be obtained, due to thick or thin ice, fast water flow, or loss of the sampling equipment in a river at spate.

A bloom of filamentous algae was collected from Thames River water in the University grounds upon August 17, 1982. Microscopic examination revealed the presence of Draparnalida glomerata (Fig. 3.1), Euglena sp. (division Euglenophycophyta), some diatoms and Spirogyra sp. containing intracellular crystals with a "cross" shape. The algae were comminuted in a mortar and pestle, then centrifuged to disrupt the cellular structure. Individual crystals were "fished out" for further analysis by hand picking under the microscope with a stainless steel needle (see section 3.6).

3.3 The upper Thames River, geographical and geological setting

The Thames River drainage basin encompasses an area of 5,000 km^2 within the peninsular of Southern Ontario, which

projects between Lake Huron on the north and Lake Erie to the south. The Thames River rises in Perth and Oxford counties, carrying its water about 300 km WSW to Lake St. Clair. The upper Thames watershed comprises the drainage area above the confluence of Dingman Creek with the main river, 16 km southwest of London: it has an area of 3,400 km^2 (Fig. 3.1).

The north Thames River rises about 16 km north of Mitchell: two additional branches, the Thames which rises near Tavistock and the middle Thames which rises in the environs of Embro, join 8 km southwest of Ingersol. The latter branch and the north Thames River confluence within the city of London (Fig. 3.1). The most comprehensive information on this drainage basin is embodied in the Upper Thames Valley Conservation Report (1952), which covers land use, forestry, hydraulics, wildlife, recreation, etc.

3.3.1 Geology of the upper Thames River drainage basin

Peninsular Southern Ontario is largely covered by a mantle of Pleistocene ($\leq$ 2 Ma) glacial tills, which average 30 m in thickness and extend to depths of 75 m. The bedrock is composed of 900 to 1,100 m of lower Palaeozoic shales and carbonates, which in turn overlie Precambrian basement.

Carbonates of the Devonian Norfold formation underlie most of the Upper Thames drainage basin: shales of the

Devonian Hamilton formation and Silurian dolomitic limestones are distributed on the northeast and southwest periphery respectively. These lower Paleozoic rocks are significant inasmuch as they constitute the provenance for the Pleistocene tills, as well as Precambrian basement rocks located to the north. Soils vary widely in type and permeability, depending on the parent material upon which they have developed. The distribution of soil types in the Thames River drainage basin is summarized in the Upper Thames Valley Conservation Report (1952).

Drainage patterns are essentially dictated by landforms which are in turn largely the product of Pleistocene glacial processes. The physiography of Southern Ontario is comprehensively reviewed by Chapman and Putnam (1966).

3.3.2 Climate

Annual precipitation averages 83 mm over Southern Ontario, with localised highs such as London which receives an annual mean of 96 mm. Rainfall over the six summer months is 46 mm, with heavy rainfall more frequent in the late summer or fall months. This temporal variation in precipitation results in two peaks in discharge rate for the Thames River - one during thawing and a second in September-December (Upper Thames Valley Conservation Report, 1952). Precise timing of peak flow depends on weather conditions of the individual year: this aspect of

the river system is considered further below.

3.4 Dissolved uranium in Thames River waters

The uranium solute concentration for Thames River waters collected monthly over a one year duration is reported in Table 3.1, for the twenty four sampling stations illustrated in Fig. 3.1. A remarkably small degree of variation is present in aqueous uranium levels (Fig. 3.2), with a grand average over all months and sampling stations of 1.46 ppb $\pm$ 0.61 1σ. This figure represents about twice the global average river dissolved uranium abundance of 0.6 ppb (Bloch, 1981). Minimum and maximum recorded U concentrations were 0.1 and 3.7 ppb respectively (Table 3.1).

During the first seven months of sampling an aliquot of the two litre volumes collected, and preconcentrated by evaporation, was also analysed for U by means of neutron activation delayed neutron counting (NADNC). In general, the results obtained by fluorimetry and delayed neutron counting (see Appendix I) are in close agreement, with the latter method yielding 80% of the results to within 10% of those obtained by flourimetry (Fig. 3.3).

3.4.1 Seasonal variations

In order to assess possible temporal variations of dissolved uranium, the data from Table 3.1 are graphically

Figure 3.2 Abundance of dissolved uranium in Thames River waters and its tributaries, averaged over the 12 month interval November 1981 to October 1982. Upper bold figures represent the sample site, lower numbers are uranium in ppb.

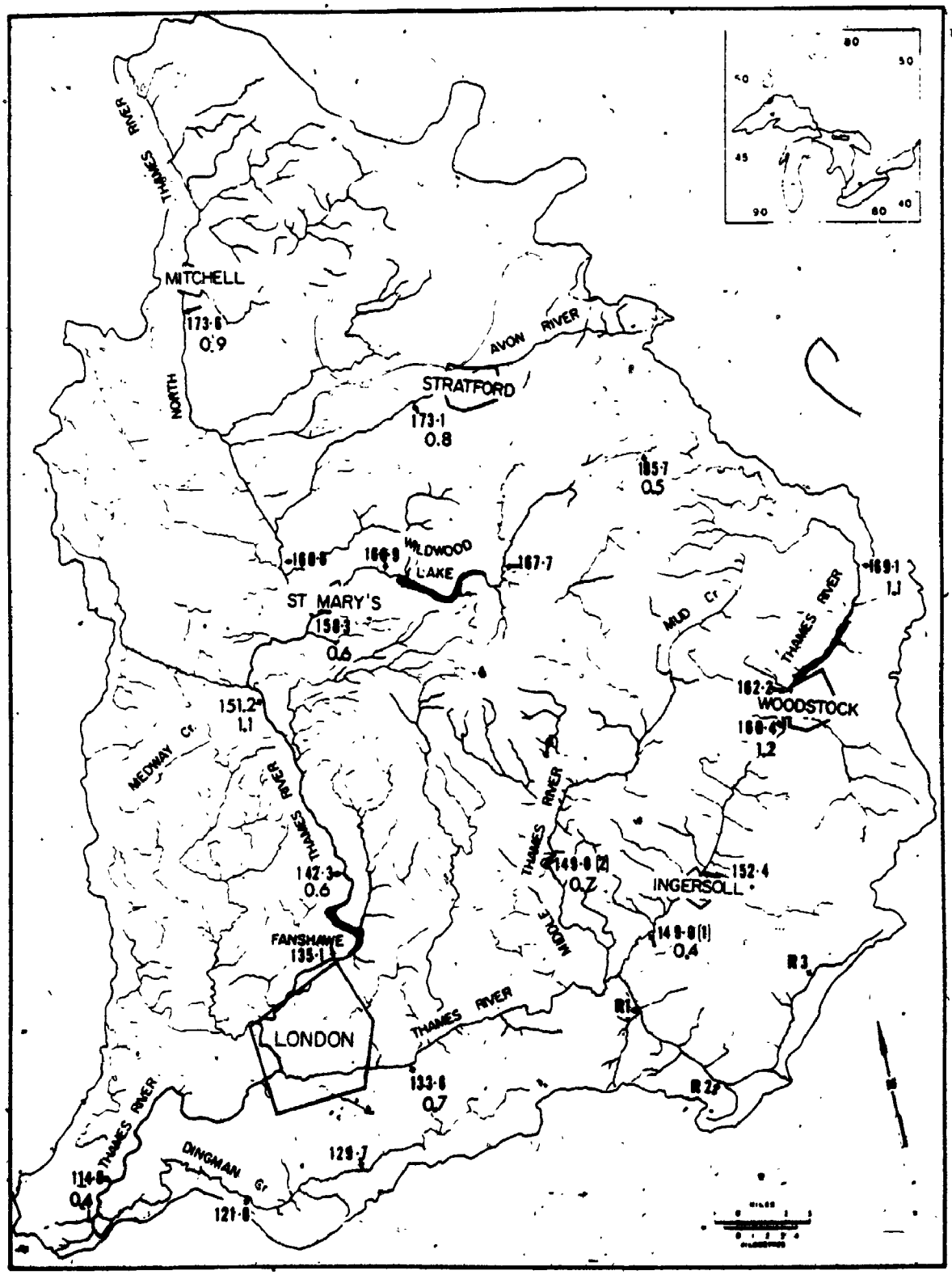
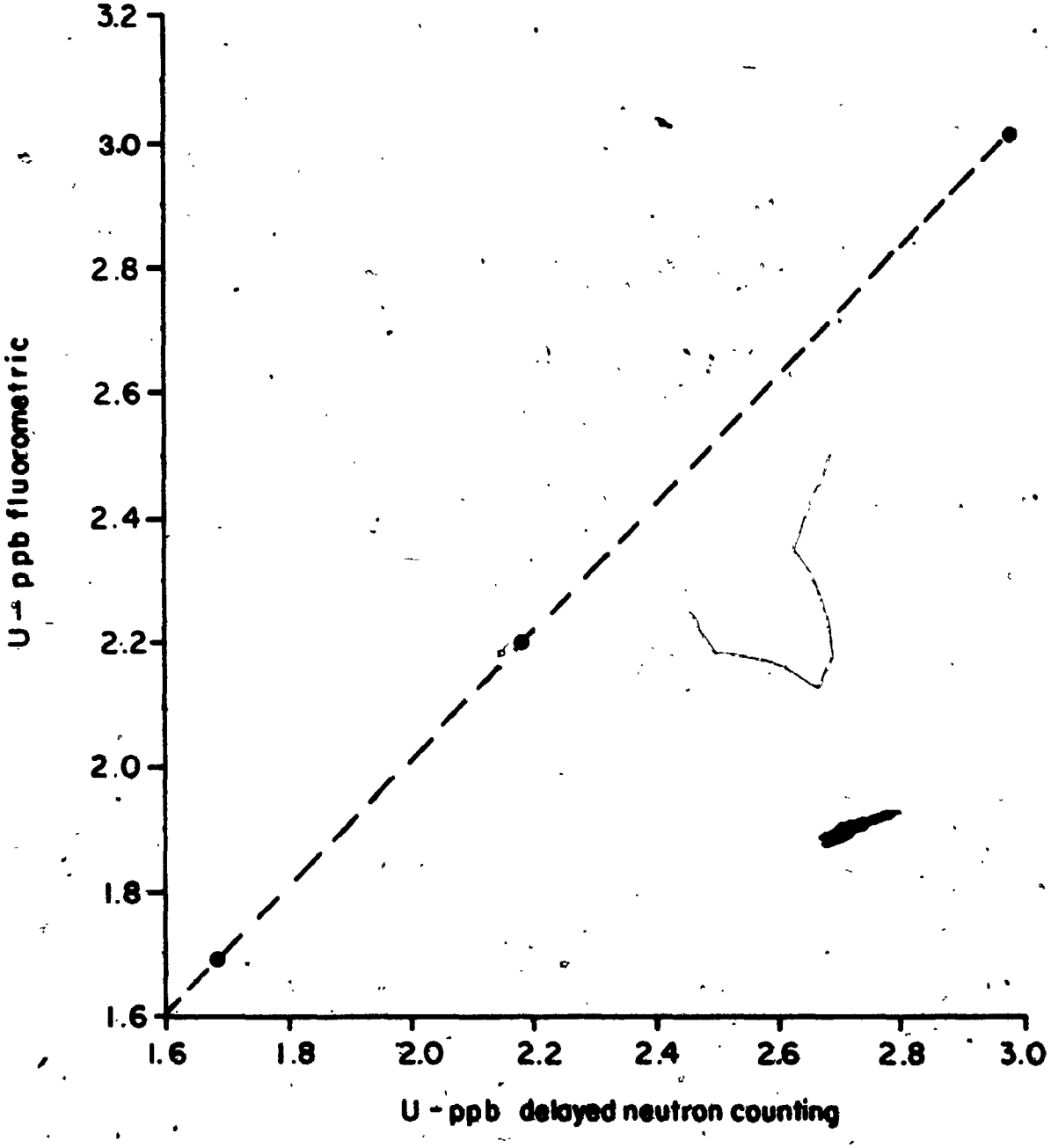

MITCHELL
173·6
0·9
NORTH THAMES RIVER
AVON RIVER
STRATFORD
173·1
0·8
185·7
0·5
166·8
166·8
WILDWOOD LAKE
167·7
ST MARY'S
158·3
0·6
169·1
1·1
MUD Cr.
THAMES RIVER
162·2
WOODSTOCK
166·6
1·2
151·2
1·1
MEDWAY Cr.
THAMES RIVER
142·3
0·6
FANSHAWE
135·1
THAMES RIVER
MIDDLE
149·8 (2)
0·2
152·4
INGERSOLL
149·8 (1)
0·4
R3
R1
R2
LONDON
THAMES RIVER
133·8
0·7
129·7
THAMES RIVER
114·8
0·4
DINGMAN Cr.
121·6

Table 3.1. Abundance of uranium in Thames river waters measured over a twelve month duration, at specified sampling stations, and reported as ppb U.† U determined fluorometrically, and by delayed neutron counting (figures in parentheses).

Sampling station	1981		1982									
	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.
173.6	2.1 (1.96)	2.6 (2.34)	2.1 (2.14)	2.6 (2.41)	1.7 (1.43)	1.0 (1.10)	0.9 (0.60)		1.3	0.9	3.1	3.2
173.1	1.3	1.2 (1.39)	1.5 (1.32)	1.6 (1.02)	1.1 (1.11)	1.0 (1.05)	1.4 (1.20)		0.6	0.8	0.8	1.4
160.8	1.9 (1.92)	1.8 (1.90)										
167.7	0.1 (0.24)	0.9 (0.91)										
160.9	0.9	0.9 (0.89)		1.5								
158.3	2.0 (1.85)	1.0 (1.11)	1.3 (1.63)	1.9 (1.87)	1.4 (1.39)	1.5 (1.54)	0.9 (1.13)	1.3	1.0	0.6	1.5	2.0
156.0	2.1	2.2 (1.58)										
151.2	2.1 (1.85)	1.8 (1.87)	1.5 (1.52)	2.0 (1.85)		1.4 (1.15)	0.4 (0.58)	1.2	0.9	1.1	0.9	1.7
142.3	1.7 (1.86)	1.0 (1.19)	1.5 (1.22)	2.3 (2.10)	1.0 (0.73)	1.0 (1.27)	1.2 (1.04)	1.1	1.1	0.6	1.4	2.2
135.1	1.7 (1.54)	1.8 (1.70)	2.1 (1.57)	2.1 (1.91)	1.3 (1.15)	0.7 (1.15)	1.5 (1.16)	1.1	1.1		1.2	2.0
185.7	0.9 (0.78)									0.5		
169.1	1.6 (1.55)	1.8 (1.65)	2.1 (1.95)	2.0 (1.83)	0.9 (0.56)	1.1 (1.11)	1.4 (1.10)		1.1	1.1	2.2	1.9
162.2	1.8 (1.85)	1.9 (2.18)										
160.4	2.6 (2.36)	1.8 (1.33)	1.6 (1.65)	1.9 (1.30)	1.1 (0.60)		0.9 (0.93)	0.9	0.8	1.2	1.8	2.0
152.4	1.7 (1.69)	0.9 (1.22)										
149.0(1)	1.2 (1.30)	1.5 (1.44)	1.8 (1.53)	2.0 (1.90)	1.3 (0.57)	1.4 (1.20)	0.8 (0.76)	0.9	1.0	0.4	1.9	1.9
149.0(2)	0.8 (0.88)	1.1 (1.39)	1.6 (1.50)	1.1 (0.77)	0.9 (0.57)	1.0 (0.88)	0.4 (0.45)		0.9	0.7	1.7	1.7
May 3	2.2 (2.18)	2.3 (2.38)										
May 2	2.7 (2.29)	3.1 (3.31)										
May 1	1.7 (1.53)	3.0 (2.93)		3.6								
133.6	1.4 (1.45)	1.5 (1.37)				1.8	1.5	1.0	1.0	0.7	2.6	
114.8	1.2 (1.39)	1.4 (1.24)	1.5 (1.25)	1.8 (1.60)	0.8 (0.58)	1.5 (1.15)	1.3 (0.91)	1.0	1.0	0.4	2.1	1.5
129.7	1.8 (2.31)	2.5 (2.59)										
121.8	1.6 (1.99)	1.9 (1.72)										

† for map illustrating location of sampling stations see Fig. 3.1.

Figure 3.3 Abundance of dissolved uranium in Thames River waters, determined by fluorimetry (vertical axis) and by delayed neutron counting (horizontal axis). Source of data Table 3.1. Dashed line corresponds to a slope of +1.


3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
U - ppb fluorometric
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
U - ppb delayed neutron counting

depicted as a function of time, for eleven of the sampling stations for which data are most complete (Fig. 3.4). A general trend is observed of higher uranium levels for the fall and winter months September through February, contrasting with depressed levels during the spring and summer months of March through August.

Generally, the seasonal trends move in harmony for the different sampling sites (Fig. 3.4), suggesting that the causal effect is operating over the entire drainage basin. Minor exceptions to the overall trends were noted: for instance uranium levels peaked in January at site 149.0(2), as against February for all the other sampling stations (Table 3.1).

A crucial question is what controls the observed temporal variation of dissolved uranium? In order to explore possible causal relationships, data for discharge rates over a calendar year, at representative locations throughout the upper Thames River system, were plotted. These data are given for 1981 (Fig. 3.5) and for 1982 (Fig. 3.6) below Fanshawe dam.

Variations in discharge rate through the year possess a characteristic profile, which is uniform from site to site (Fig. 3.5). A January (1981) minima is succeeded by a peak over February and March 1981, with an ensuing continuous decline in discharge rate to July. This July low is followed by a sharp rise to a September maxima, with

Figure 3.4 Seasonal variation of dissolved uranium at specified sampling sites of the upper Thames River and tributaries (for sample locations see Fig. 3.1). Solid points, fluorometric data, circles correspond to analyses by delayed neutron counting.

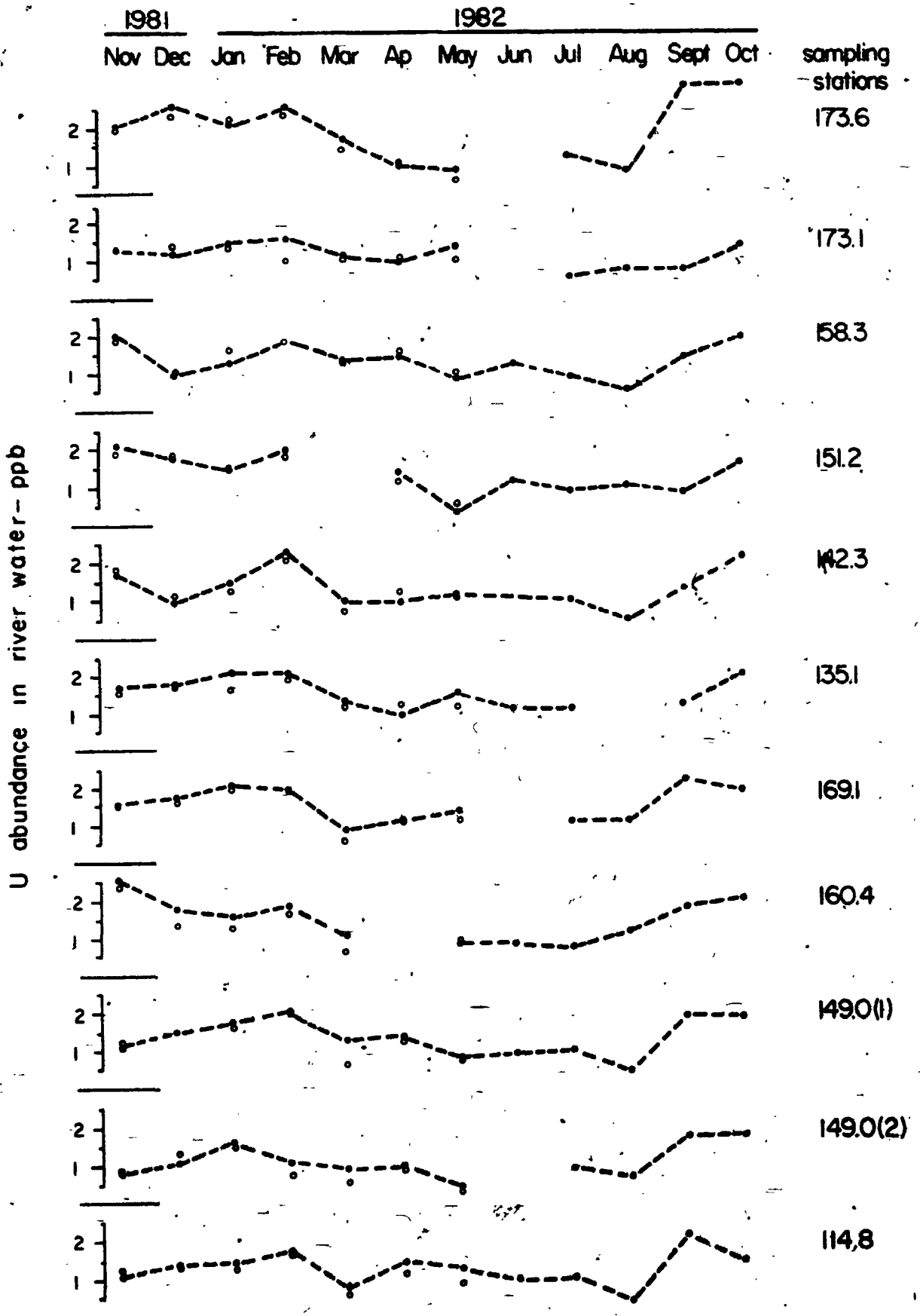

1981
1982
Nov Dec Jan Feb Mar Ap May Jun Jul Aug Sept Oct
sampling stations
U abundance in river water - ppb
2
1
173.6
173.1
158.3
151.2
142.3
135.1
169.1
160.4
149.0(1)
149.0(2)
114.8

Figure 3.5 Discharge rate at specified sites throughout the upper Thames River drainage basin, 1981 (for locations see Fig. 3.1).

A - Thamesford - station number 02GD004

B - St. Marys - station number 02GD005

C - Mitchell - station number 02GD014

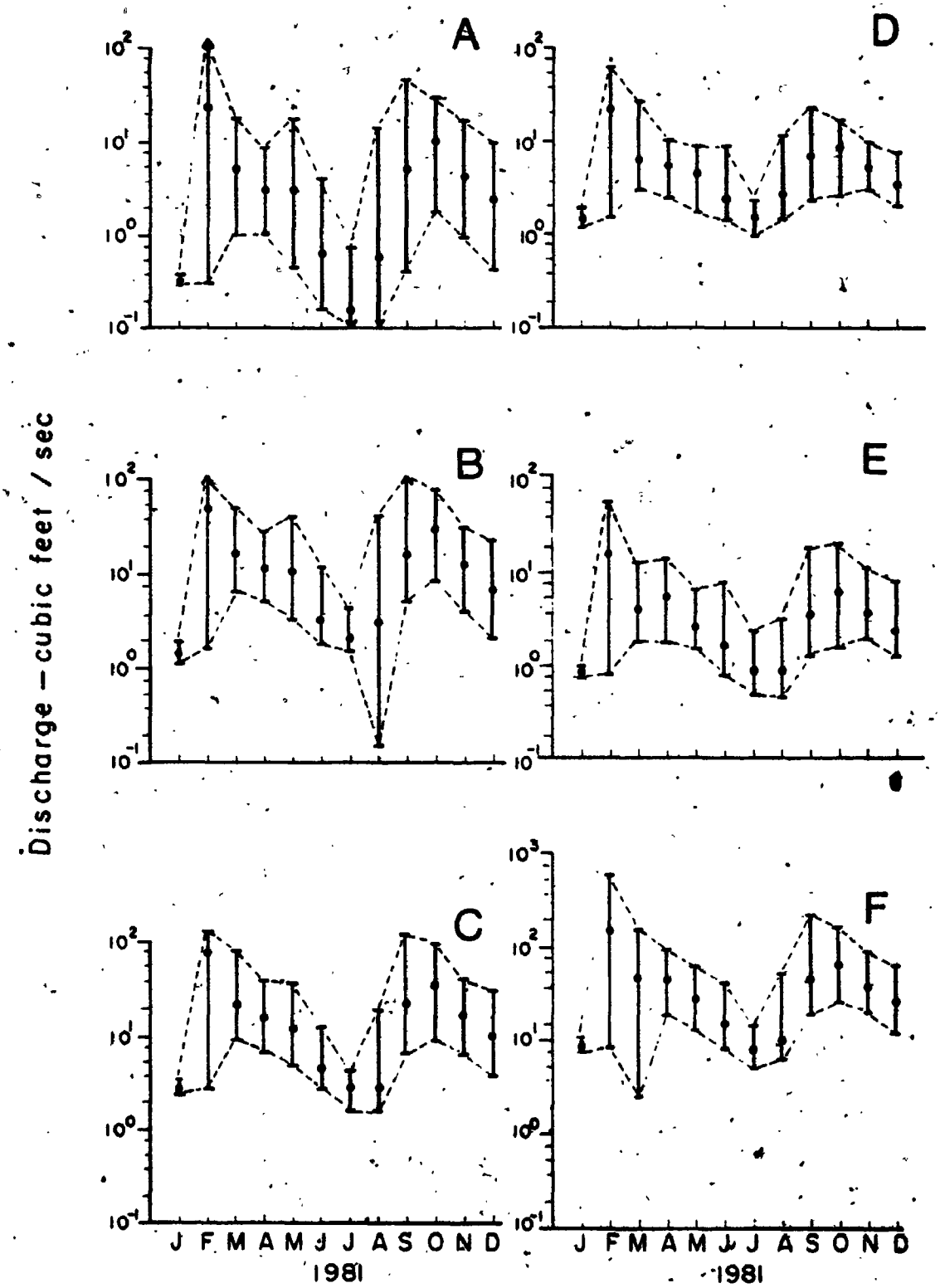
D - Byron - station number 02GE002

E - Ingersoll - station number 02GD016

F - below Fanshawe dam - station number 02GD003

Solid circles represent the average discharge, bars the maximum and minimum for the month, and arrows, data off scale.

Source of data - Inland water directorate, Ministry of Supply and Services, Canada.


A
B
C
D
E
F
Discharge — cubic feet / sec
J F M A M J J A S O N D
1981

rates declining thereafter through December. The February peak corresponds to thawing, whereas the surge in September month correlates with increased rainfall. In 1982 the thawing surge was displaced to March plus April, and the September-October peak of 1981 replaced by a continuous rise through December (Fig. 3.6).

In some respects a gross covariance exists between discharge rate and dissolved uranium, with both parameters relatively lower for March through August, followed by a common upturn over September and fall. However the January-February discharge low is not tracked by uranium, which is relatively higher at that time of the year.

An intriguing feature of these data is that whereas variations in discharge rate of 1.5 to 2 orders of magnitude occur over a calendar year, excursions in aqueous uranium abundance, even where sympathetic with discharge, are at most a factor of two (i.e. the U concentration is essentially constant). Thus, for the March through October interval elevated discharge rates do not dilute uranium proportionately. Rather, increased throughput of water in the soil/groundwater system, reflected in greater river discharge, is more than compensated for by increased release of uranium to the water, in order to account for the aqueous uranium maxima.

These data are collectively interpreted to indicate that the uranium source is close to solution equilibrium

Figure 3.6 Discharge rate below Fanshawe Lake dam for 1982 (for location see Fig. 3.1). Circles represent mean discharge, bars the monthly maximum and minimum.

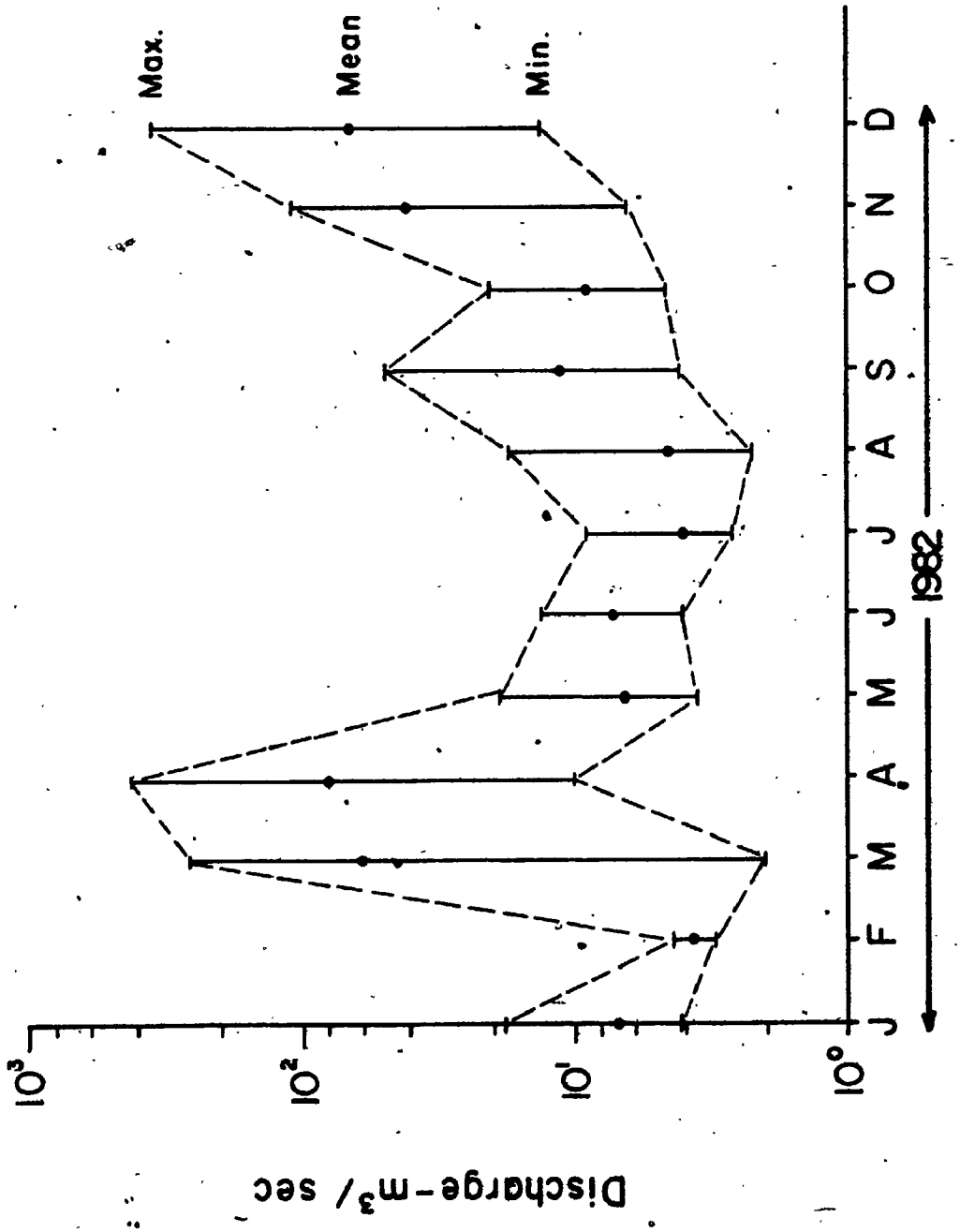

Max.
Mean
Min.
J F M A M J J A S O N D
1982
10^3
10^2
10^1
10^0
Discharge-m^3/ sec

Figure 3.7 Abundance of solid and dissolved components transported by river waters of the upper Thames River drainage basin at specified sites, for the interval November 1981 through December 1982. Suspended particulates (diamonds), dissolved components (open circles), plus the total (closed circles). For location of sample sites see Fig. 3.1.

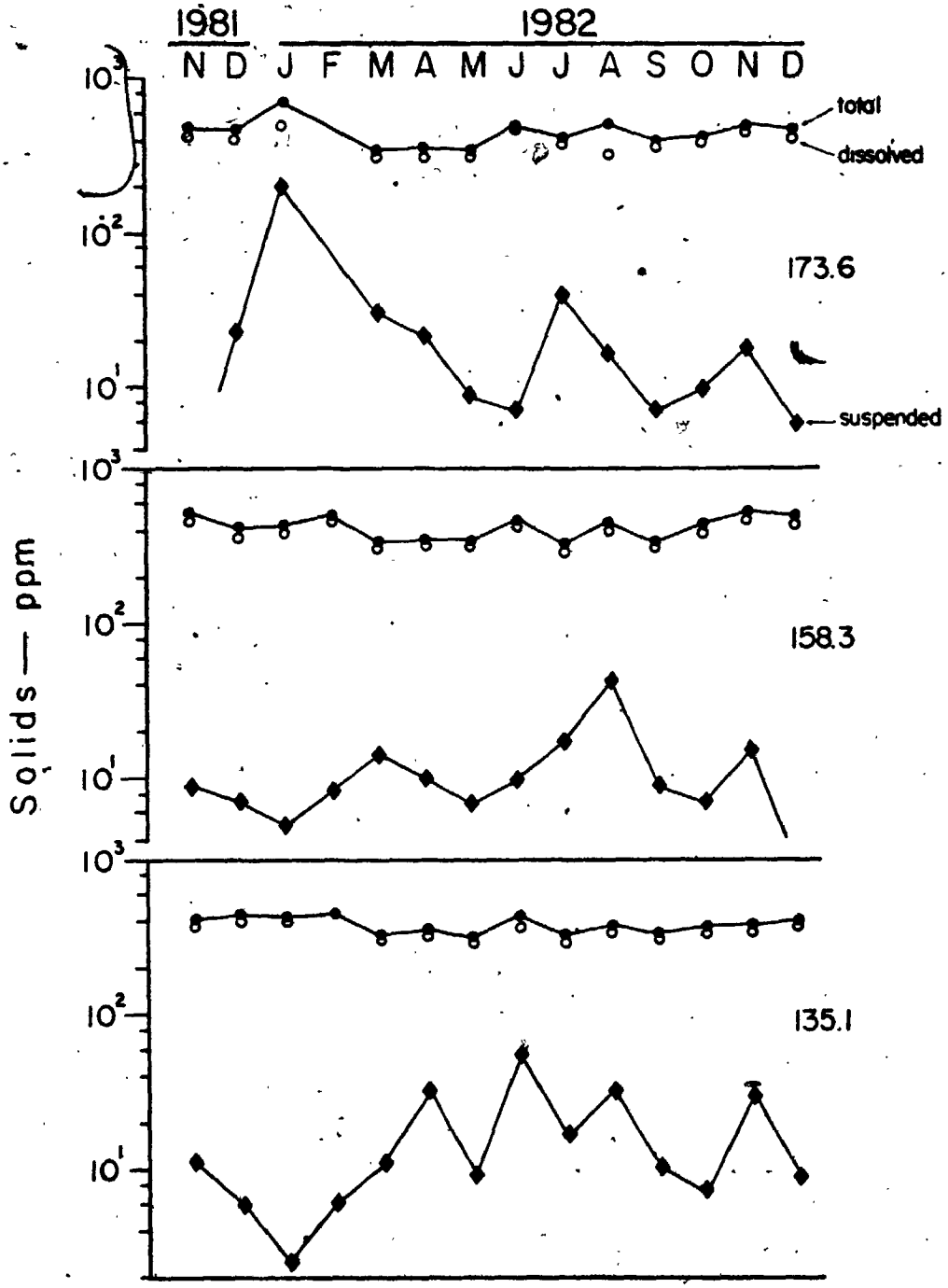

1981
1982
N D J F M A M J J A S O N D
total
dissolved
suspended
173.6
158.3
135.1
Solids — ppm
10^3
10^2
10^1

Figure 3.8 Abundance of solid and dissolved components transported by river waters of the upper Thames River drainage basin at specified sites, for the interval November 1981 through December 1982. Suspended particulates (diamonds), dissolved components (open circles), plus the total (closed circles). For location of sites see Fig. 3.1.

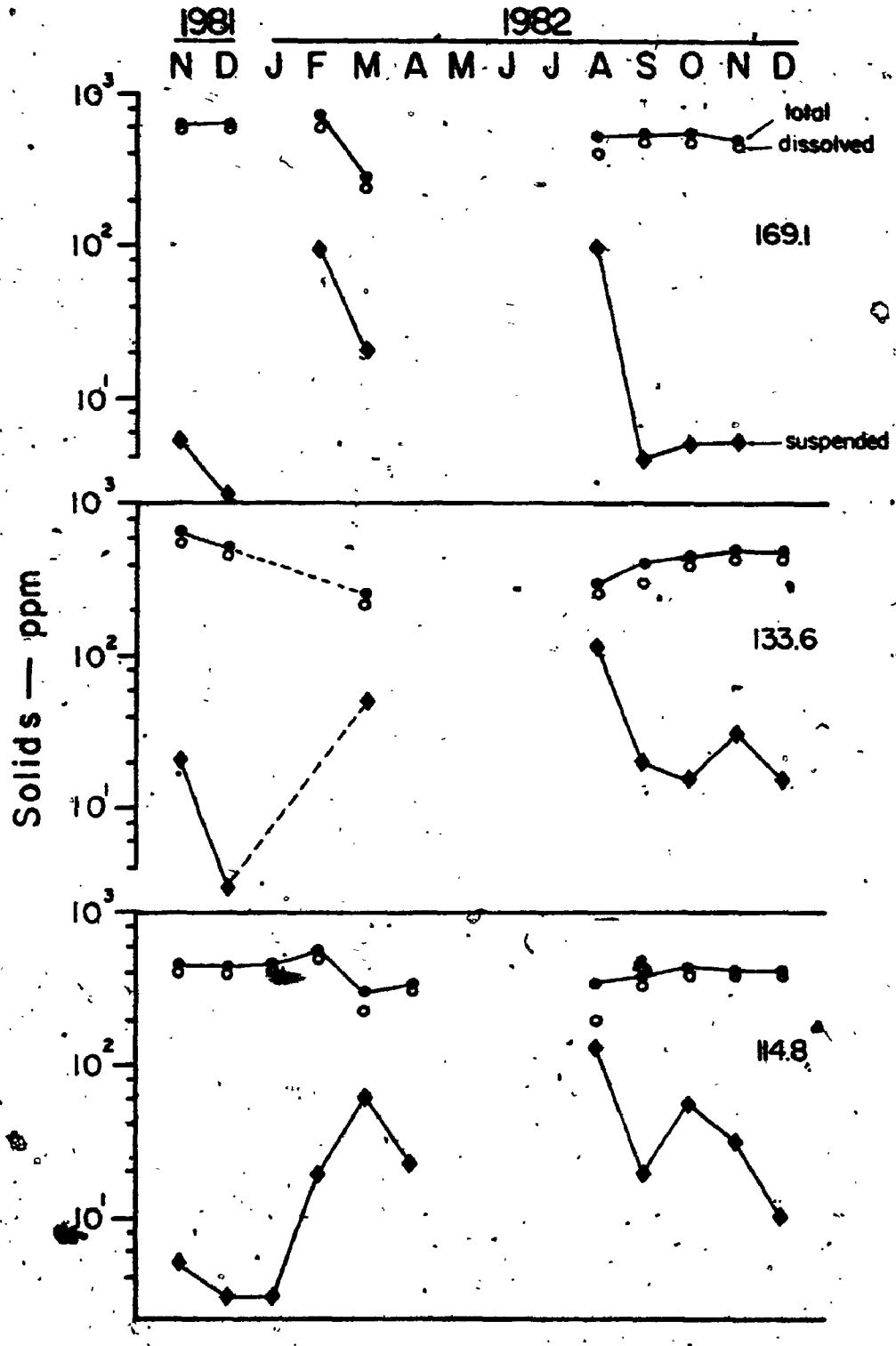

1981
1982
N D J F M A M J J A S O N D
total
dissolved
suspended
169.1
133.6
114.8
Solids — ppm
10^3
10^2
10^1

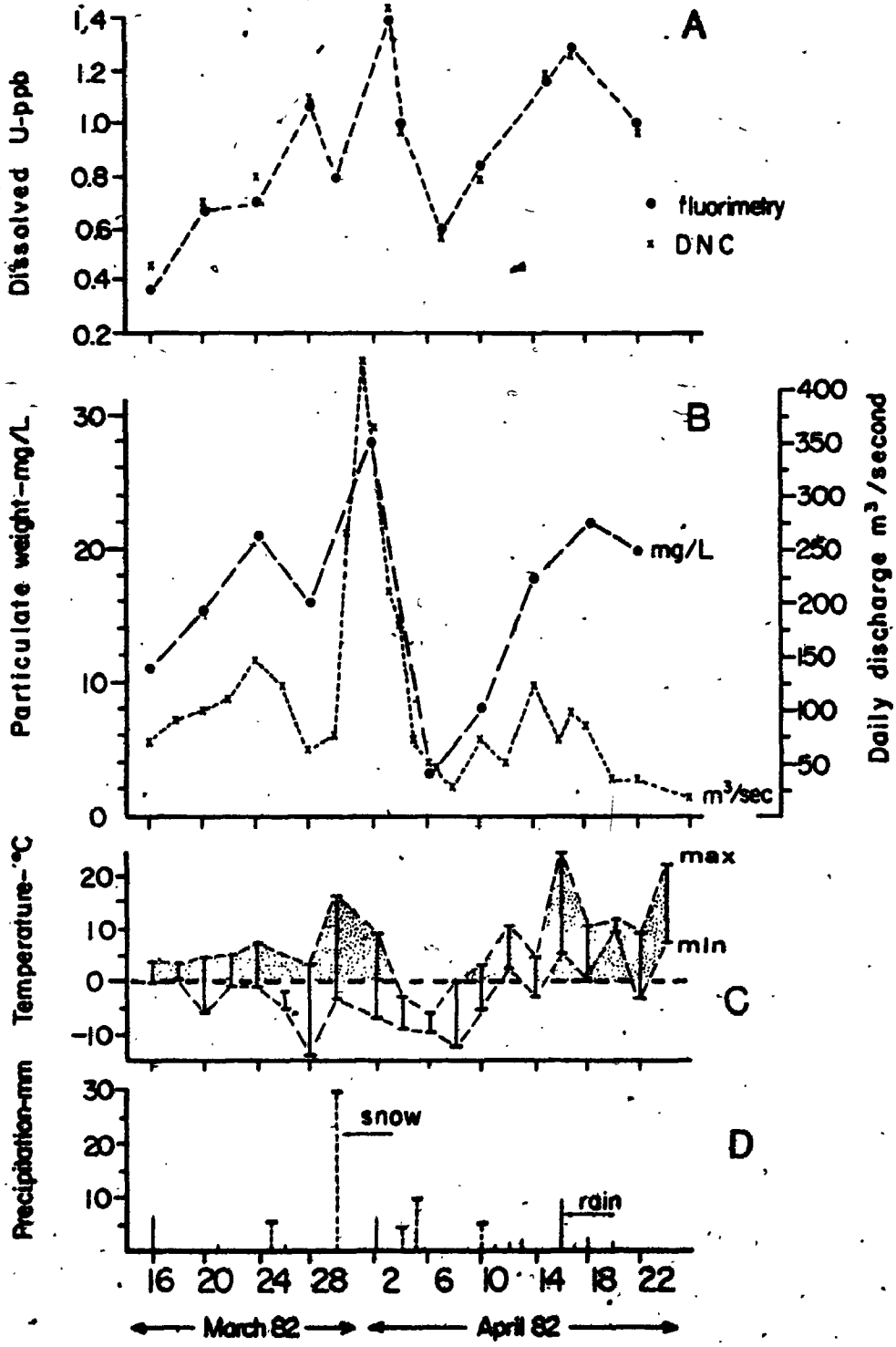
Figure 3:9 Characteristics of Thames River waters, at the University grounds, during thawing March plus April 1982.

A. Abundance of dissolved uranium.

B. Total weight of acid soluble >0.45 μm suspended particulates (this study). Discharge rate is for Fanshawe dam - source of data. Water Survey of Canada, station 02GD003, Ministry of the Environment.

C. Maximum and minimum daily temperatures for specified days, during March and April 1982.

D. Amount of precipitation for specified days of March and April 1982. Solid bars represent rainfall, dashed bars snow.


A
Dissolved U-ppb
1.4
1.2
1.0
0.8
0.6
0.4
0.2
fluorimetry
DNC
B
Particulate weight-mg/L
30
20
10
0
mg/L
m³/sec
Daily discharge m³/second
400
350
300
250
200
150
100
50
C
Temperature-°C
20
10
0
-10
max
min
D
Precipitation-mm
30
20
10
snow
rain
16 20 24 28 2 6 10 14 18 22
March 82
April 82

with water passing through the soil, and into the river drainage system. This effect is also observed for the total dissolved component of Thames River waters which is remarkably uniform, at 500 ppm throughout the year, independent of river flow rate (Figs. 3.7, 3.8).

3.4.2 Behaviour during spring thawing

Concentrations of dissolved uranium in Thames River water were monitored at four day intervals during the end of the 1981-1982 winter, for the purpose of assessing the possible influence of thawing. Two litre aliquots were collected from the river Thames in the University grounds commencing 16th March 1982 and terminating 26th April 1982. The results are portrayed graphically in Fig. 3.9, along with particulate weight, discharge rate, daily temperatures and precipitation.

Two maxima of aqueous uranium were recorded on the 2nd April (1.4 ppb) and 18th April (1.3 ppb), respectively. The profiles of uranium abundance, particulate weight and discharge covary; these in turn clearly relate to higher daily temperatures rather than to a greater incidence of precipitation, implying that the uranium peak is largely indigenous to a transient meltwater surge. The observed correlation of particulate weight with discharge rate is as expected, given that the suspended particulate load of rivers is a positive function of water velocity.

Dissolved uranium concentrations of 0.4 to 1.0 ppb for March 16-28 are generally slightly lower than the value of 1.3 ppb recorded for this month at Fanshawe dam (station 135.1), and all other sampling stations during March (Table 3.1). This is the obvious uranium peaks of 26th March and 28 March through April 3. Such a result may have been a fortuitous coincidence of sampling the river system as a whole (Table 3.1) during the thawing induced uranium peaks of late March, and would imply shorter time-scale variabiling in dissolved uranium than is indicated in Table 3.1 and Fig. 3.4, but probably only during thawing.

3.5 The uranium complement of riverine suspended particulates

Uranium abundances in acid (HNO_3 + $HClO_4$) soluble suspended particulates, along with particulate weight are recorded in Tables 3.2 through 3.13, and a summary of all results is presented in Table 3.14. Suspended particulates of >0.45 μm were recovered during filtration of the 2 litre aliquots of river waters as described in Appendix I. This 'screen' size retained mineral particulates, unicellular algae, some filamentous algae, protozoa, some bacteria, and yeast cells, but probably not virus: the relative proportion of inorganic mineral particulates to microorganisms in the suspended fraction is considered further below. Particulate weights and their uranium contents, averaged

Table 3.2. Abundance of dissolved uranium in Thames river waters, and of uranium in >0.4 μm suspended particulates, at specified sampling stations, November, 1981. Analyses by fluorimetry.

Sampling station	U-ppb river	U-ppb >0.4 μm particulates	Particulate weight-mg	Concentration factor particulates/water
173.6	2.07	<30,000	0.8	
173.1	1.26	5,000	5.0	4,000
160.8	1.95	6,300	4.0	3,200
167.7	0.15	<13,000	1.9	
160.9	0.89	670	37.1	750
158.3	2.04	7,800	3.2	3,800
156.0	2.14	11,000	2.3	5,100
151.2	2.07	<7,000	3.4	
142.3	1.71	12,000	2.1	7,000
135.1	1.70	2,900	8.7	1,700
185.7	0.82	<5,000	4.8	
169.1	1.61	<8,000	3.1	
162.2	1.83	2,200	11.4	1,200
160.4	2.60	4,200	6.0	1,600
152.4	1.73	<5,000	5.0	
149.0 (1)	1.16	43,000†	2.3	37,000
149.0 (2)	0.84	8,700†	8.6	10,000
Rey 3	2.24	<4,000	5.6	
Rey 2	2.61	<4,000	6.2	
Rey 1	1.66	<2,000	10.2	
133.6	1.44	12,000†	4.3	8,300
114.8	1.19	8,000	3.1	6,700
129.7	1.85	1,700	15.0	
121.8	1.63	3,800	6.6	

† Most significant data for particulates, where U abundance in analyate >> instrumental detection limit.

Table 3.3 Abundance of dissolved uranium in Thames river waters, and of uranium in ≥0.4 μm suspended particulates, at specified sampling stations, December, 1981. Analyses by fluorimetry.

Sampling station	U-ppb river	U-ppb >0.4 μm particulates	particulate weight-mg	Concentration factor particulates/water
173.6	2.60	3,500	7.1	1,300
173.1	1.27	58,000	1.3	46,000
160.8	1.84	125,000	0.2	68,000
167.7	0.88	<12,000	2.1	
160.9	0.87	<2,000	12.3	
158.3	0.99	4,000	6.3	4,000
156.0	2.20	100,000	0.5	45,000
151.2	1.85	42,000	0.6	23,000
142.3	0.96	-	<0.1	
135.1	1.80	6,800	3.7	3,80
185.7	0.93	<16,000	1.6	
169.1	1.80	20,000	1.3	11,000
162.2	1.86	45,000†	1.1	24,000
160.4	1.80	750,000†	0.1	420,000
152.4	0.95	250,000	0.1	260,000
149.0 (1)	1.50	83,000	0.3	55,000
149.0 (2)	1.08	26,000†	1.9	24,000
Rey 3	2.35	54,000†	1.4	21,000
Rey 2	3.06	17,000†	6.6	5,500
Rey 1	2.99	56,000†	2.5	19,000
133.6	1.55	500,000	0.1	320,000
114.8	1.44	<16,000	1.6	
129.7	2.55	10,000	2.5	3,900
121.8	1.86	<18,000	1.4	

† Most significant data for particulates, where U in analyate >> instrumental detection limit.

Table 3.4 Abundance of dissolved uranium in Thames river waters, and of uranium in >0.4 μm suspended particules, at specified sampling stations, January, 1982. Analyses by fluorimetry.

Sampling station	U-ppb river	U-ppb >0.4 μm particulates	Particulate weight-mg	Concentration factor particulates/water
173.6	2.11	3,300†	114.0	1,600
173.1	11.0	173,000	1.3	16,000
158.3	1.3	35,700	4.2	27,000
15	1.5	<4,600	21.9	
142.3	1.5	<45,000	2.2	
135.1	2.1	11,400†	13.1	5,400
169.1	2.1	83,000†	9.7	39,000
160.4	1.6	65,200	2.3	41,000
149.0 (1)	1.8	27,800	3.6	15,000
149.0 (2)	1.6	13,800†	27.2	8,600
114.8	1.5	<20,400	4.9	

† Most significant data for particulates, where U in analyate >> instrumental detection limit.

Table 3.5. Abundance of dissolved uranium in Thames river waters, and of uranium in >0.4 μm suspended particulates, at specified sampling stations, February, 1982. Analyses by fluorimetry.

Sampling station	U-ppb river	U-ppb >0.4 μm particulates	Particulate weight-mg	Concentration factor particulates/water
173.6	2.6	107,000†	17.6	41,000
173.1	1.6	<53,000	1.9	<33,000
160.9	1.5	88,200	1.7	59,000
158.3	1.9	40,500	3.7	21,000
151.2	2.0	93,700	1.6	47,000
142.3	2.3	19,700†	7.6	8,600
135.1	2.1	44,600	5.6	21,000
169.1	2.0	1,500	101.8	750
160.4	1.9	73,300†	2.5	38,000
149.0 (1)	2.0	146,000	1.2	73,000
149.0 (2)	1.1	9,200†	27.2	8,400
Rey 1	3.6	24,600†	6.1	6,800
114.8	1.8	<35,000	2.8	<19,000

† Most significant data for particulates, where U in analyate >> instrumental detection limit.

Table 3.6. Abundance of dissolved uranium in Thames river waters, and of uranium in >0.4 μm suspended particulates, at specified sampling stations, March, 1982. Analyses by fluorimetry.

Sampling station	U-ppb river	U-ppb particulates	particulate weight-mg	Concentration factor particulates/water
173.6	1.7	27,600†	12.9	16,000
173.1	1.1	11,000†	29.5	10,000
158.3	1.4	204,000	1.1	146,000
142.3	1.0	41,300†	10.9	41,000
135.1	1.3	20,800†	14.4	16,000
169.1	0.9	13,900†	21.5	15,400
160.4	1.1	33,100†	13.6	30,000
149.0 (1)	1.3	18,600†	17.5	14,000
149.0 (2)	0.9	6,000†	53.2	6,800
133.6	0.5	12,900†	44.5	26,000
114.8	0.8	29,800†	75.6	37,000

† Most significant data for particulates, where U in analyate >> instrumental detection limit.

Table 3.7. Abundance of dissolved uranium in Thames river waters, and in >0.4 μm suspended particulates, at specified sampling stations, April, 1982. Analyses by fluorimetry.

Sampling station	U-ppb river	U-ppb >0.4 μm particulates	Particulate weight-mg	Concentration factor particulates/water
173.6	1.0	7,800†	19.3	7,800
173.1	1.0	3,500†	65.0	3,500
158.3	1.5	17,300†	11.4	11,000
151.2	1.4	44,400†	9.0	32,000
142.3	1.0	3,300	30.2	3,300
135.1	0.7	10,200†	39.2	15,000
169.1	1.1	<9,400	10.6	<9,000
149.0 (1)	1.4	<7,200	13.8	<5,000
149.0 (2)	1.0	17,400†	8.6	17,000
133.6	1.8	<11,100	9.0	<6,000
114.8	1.5	5,600	26.7	3.700

† Most significant data for particulates, where U in analyate >> instrumental detection limit.

Table 3.8. Abundance of dissolved uranium in Thames river waters, and of uranium in >0.4 μm suspended particulates, at specified sampling stations, May 1982. Analyses by fluorimetry.

Sampling station	U-ppb river	U-ppb >0.4 μm particulates	Particulate weight-mg	Concentration factor particulates/water
173.6	0.9	16,200†	20.1	18,000
173.1	1.4	10,600	18.8	7,600
158.3	0.9	41,700†	78.0	46,000
151.2	0.4	29,800	6.7	75,000
142.3	1.2	18,900†	13.2	16,000
135.1	1.5	63,900	4.3	43,000
169.1	1.4	38,000†	7.9	27,000
160.4	0.9	13,900	14.4	15,000
149.0 (1)	0.8	32,000†	7.8	40,000
149.0 (2)	0.4	45,900	4.9	115,000
133.6	1.5	38,000†	7.9	25,000
114.8	1.3	64,600	5.8	50,000

† Most significant data for particulates, where U in analyate >> instrumental detection limit.

Table 3.9. Abundance of dissolved uranium in Thames river waters, and of uranium in >0.4 μm suspended particulates, at specified sampling stations, June 1982. Analyses by fluorimetry.

Sampling station	U-ppb river	U-ppb >0.4 μm particulates	acid soluble particulate weight - mg[1]	residual particulate weight-mg[2]	acid soluble/ residual particulates[3]	Concentration factor particulates/water[1]
173.6	1.14	1,640	7.3	-		1,400
173.1	0.91	10,000†	3.7	4.4	0.84	11,000
158.3	1.30	12,100†	3.1	9.5	0.33	9,300
151.2	1.25	2,160†	17.1	47.5	0.36	1,700
142.3	1.14	7,600	4.7	-		6,700
135.1	1.13	<770	15.5	5.7	2.7	
169.1	1.06	5,260†	7.8	-		5,000
160.4	0.90	1,700	7.1	4.2	1.7	1,900
149.0 (1)	0.91	<1,800	9.5	6.8	1.4	
149.0 (2)	1.22	<2,300	5.1	3.3	1.5	
133.6	1.00	1,660†	22.3	54.8	0.41	1,700
114.8	1.04	2,450	10.2	37.3	0.27	2,300

† most significant data, where U abundance in analyate >> instrumental detection limit

1 acid soluble particulate weight - total dry weight of >0.4 μm suspended particulates in 2,000 ml river water, minus the weight of residual particulates, after HNO_3 and $CHlO_4$ digestion

2 residual particulate weight = weight of residual particulates after HNO_3 and $HClO_4$ attack of total suspended particulates

3 this column = weight of 1, divided by weight of 2

Table 3.10. Abundance of dissolved uranium in Thames river waters, and of uranium in >0.4 μm suspended particulates, at specified sampling stations, July 1982. Analyses by fluorimetry.

Sampling station	U-ppb river	U-ppb >0.4 μm particulates	acid soluble particulate weight-mg[1]	residual particulate weight-mg[2]	acid soluble/ residual particulates[3]	Concentration factor particulates[1]/water
173.6	1.30	<1,700	7.3	-		
173.1	0.61	<2,500	4.9	3.2	1.5	
158.3	1.0	<1,900	6.6	5.5	1.2	
151.2	0.96	<1,200	10.5	20.4	0.51	
142.3	1.18	6,060	3.3	4.6	0.72	5,100
135.1	1.14	6,940†	10.8	3.0	3.6	6,100
169.1	1.11	9,760†	4.1	9.3	0.44	8,800
160.4	0.83	<2,700	4.6	4.5	1.0	
149.0 (1)	1.0	<1,200	10.7	5.0	2.1	
149.0 (2)	0.96	3,570	5.6	3.8	1.5	3.700
133.6	1.0	<900	14.0	7.2	1.9	
114.8	1.0	2,170	9.2	6.1	1.5	2,170

† most significant data, where U abundance in analyte >> instrumental detection limit

1 acid soluble particulate weight - total dry weight of >0.4 μm suspended particulates in 2,000 ml river water, minus the weight of residual particulates, after HNO_3 and $CHlO_4$ digestion

2 residual particulate weight = weight of residual particulates after HNO_3 and $HClO_4$ attack of total suspended particulates

3 this column = weight of 1, divided by weight of 2

Table 3.11. Abundance of dissolved uranium in Thames river waters, and of uranium in >0.4 μm suspended particulates, at specified sampling stations, August 1982. Analyses by fluorimetry.

Sampling station	U-ppb river	U-ppb >0.4 μm particulates	acid soluble particulate weight - mg[1]	residual particulate weight - mg[2]	acid soluble/ residual particulates[3]	Concentration factor particulates/water
173.6	0.9	5,600	160.9	25.9	6.2	6,200
173.1	0.8	<1,900	15.5	5.5	2.8	
158.3	0.6	<3,200	9.5	17.5	0.54	
151.2	1.1	4,000	95.3	1,049.5	0.09	3,600
142.3	0.6	<1,500	20.7	28.8	0.72	
185.7	0.5	9,800	35.4	49.1	0.72	20,000
169.1	1.1	880	68.0	4.2	16	800
160.4	1.2	91,000	1,023	2.4	430	76,000
149.0(1)	0.4	<1,000	30.1	30.6	0.98	
149.0(2)	0.7	<3,000	10.3	8.4	1.2	
133.6	0.7	<1,100	27.8	69.6	0.40	
114.8	0.4	<1,100	28.2	164.0	0.17	

† most significant data, where U abundance in analyte >> instrumental detection limit
1 acid soluble particulate weight = total dry weight of >0.4 μm suspended particulates in 2,000 ml river water, minus the weight of residual particules, after HNO_3 and $HClO_4$ digestion.
2 Residual particulate weight = weight of residual particulates after HNO_3 and $HClO_4$ attack of total suspended particules.
3 this column = weight of 1, divided by weight of 2

Table 3.12. Abundance of dissolved uranium in Thames river waters, and of uranium in >0.4 μm suspended particulates, at specified sampling stations, September 1982. Analyses by fluorimetry.

Sampling station	U-ppb river	U-ppb >0.4 μm particulates	acid soluble particulate weight-mg[1]	residual particulate weight-mg[2]	acid soluble/ residual particulates[3]	Concentration factor particulates/water
173.6	3.1	28,600†	1.4	3.9	0.36	2,800
173.1	0.84	50,000†	1.5	3.9	0.38	59,000
158.3	1.51	8,330	2.4	3.8	0.63	5,500
151.2	0.96	275,000†	0.2	2.5	0.08	290,000
142.3	1.44	10,500†	3.8	3.7	1.0	7,300
135.1	1.20	42,300†	1.3	0.6	2.2	35,000
169.1	2.26	2,820	7.1	-		1,200
160.4	1.83	1,960	10.2	6.9	1.5	1,100
149.0 (1)	1.95	1,450†	27.5	8.1	3.4	700
149.0 (2)	1.75	26,700	1.5	-		15,000
133.6	2.61	5,880†	6.8	2.9	2.3	2,200
114.8	2.18	7,690	2.6	3.4	0.76	3,500

† most significant data, where U abundance in analyate >> instrumental detection limit

1 acid soluble particulate weight = total dry weight of >0.4 μm suspended particulates in 2,000 ml river water, minus the weight of residual particulates, after HNO_3 and $HClO_4$ digestion.

2 residual particulate weight = weight of residual particulates after HNO_3 and $HClO_4$ attack of total suspended particulates.

3 this column = weight of 1, divided by weight of 2

Table 3.13. Abundance or uranium in Thames river waters, and of uranium in >0.4 μm suspended particulates, at specified sampling stations, October, 1982. Analyses by fluorimetry.

Sampling station	U-ppb river	U-ppb >0.4 μm particulates	acid soluble particulate weight - mg[1]	residual particulate weight - mg[2]	acid soluble/ residual particulates[3]	Concentration factor particulates/water
173.6	3.2	78,600	0.7	1.2	8.6	24,000
173.1	1.4	<20,000	0.6	-		
158.3	2.0	7,140	5.6	7.6	0.74	3,600
151.2	1.7	9,300	4.3	1.0	4.3	5,500
142.3	2.2					
135.1	2.0	25,000	0.8	2.8	2.8	12,000
169.1	1.9	10,300	3.9	5.6	0.70	5,400
160.4	2.0	7,550	5.3	9.9	0.53	3,800
149.0(1)	1.9	9,020	6.1	3.5	1.7	4,700
149.0(2)	1.7	13,300	1.5	8.5	0.18	770
114.8	1.5	2,630	1.9	22.9	0.08	1,800

† most significant data, where U abundance in analyte >> instrumental detection limit

1 acid soluble particulate weight = total dry weight of >0.4 μm suspended particulates in 2,000 ml river water, minus the weight of residual particules, after HNO_3 and $HClO_4$ digestion.

2 residual particulate weight = weight of residual particules after HNO_3 and $HClO_4$ attack of total suspended particulates.

3 this column = weight of 1, divided by weight of 2

Table 3.14. Summary of data for uranium abundance in Thames river waters and suspended particulates, along with particulate weight, for the twelve months, November 1981 to October 1982, averaged over all sampling stations (see Tables 3.1 to 3.13 and Fig. 3.1).

Month 1981/82	U-ppb - waters			U-ppb >0.4 μm soluble particulates[1]			particulate weight-soluble, mg[2]			Total particulate weight - mg[3]		
	average	1σ	n	average	1σ	n	average	1σ	n	average	1σ	n
November	1.66	(0.58)	24	15,900	(31,000)	15	6.7	(7.3)	24			
December	1.70	(0.66)	24	117,000	(199,000)	18	3.7	(7.6)	24			
January	1.69	(0.29)	11	44,300	(58,000)	8	18.6	(32.7)	11			
February	2.03	(0.56)	13	58,900	(46,000)	11	13.9	(27.0)	13			
March	1.15	(0.27)	10	34,800	(57,600)	11	13.4	(11)	11			
April	1.22	(0.32)	11	13,700	(13,600)	8	11.1	(8.6)	11			
May	1.05	(0.39)	12	34,500	(18,000)	12	8.0	(10.1)	12			
June	1.06	(0.13)	12	4,950	(4,032)	9	9.4	(2.9)	12	11.9	(12.5)	12
July	0.98	(0.17)	12	5,700	(3,000)	5	3.8	(1.6)	12	6.8	(3.4)	12
August	0.75	(0.27)	12	5,900	(80)	5	23.2	(23)	11	40.5	(32)	12
September	1.77	(0.67)	12	38,400	(76,300)	12	2.7	(3.7)	11	4.4	(4.6)	12
October	1.95	(0.47)	11	18,700	(23,500)	9	1.6	(1.1)	10	4.7	(3.6)	10

1 This column refers to the abundance of uranium in >0.4 μm particulates soluble in HNO_3 + $HClO_4$

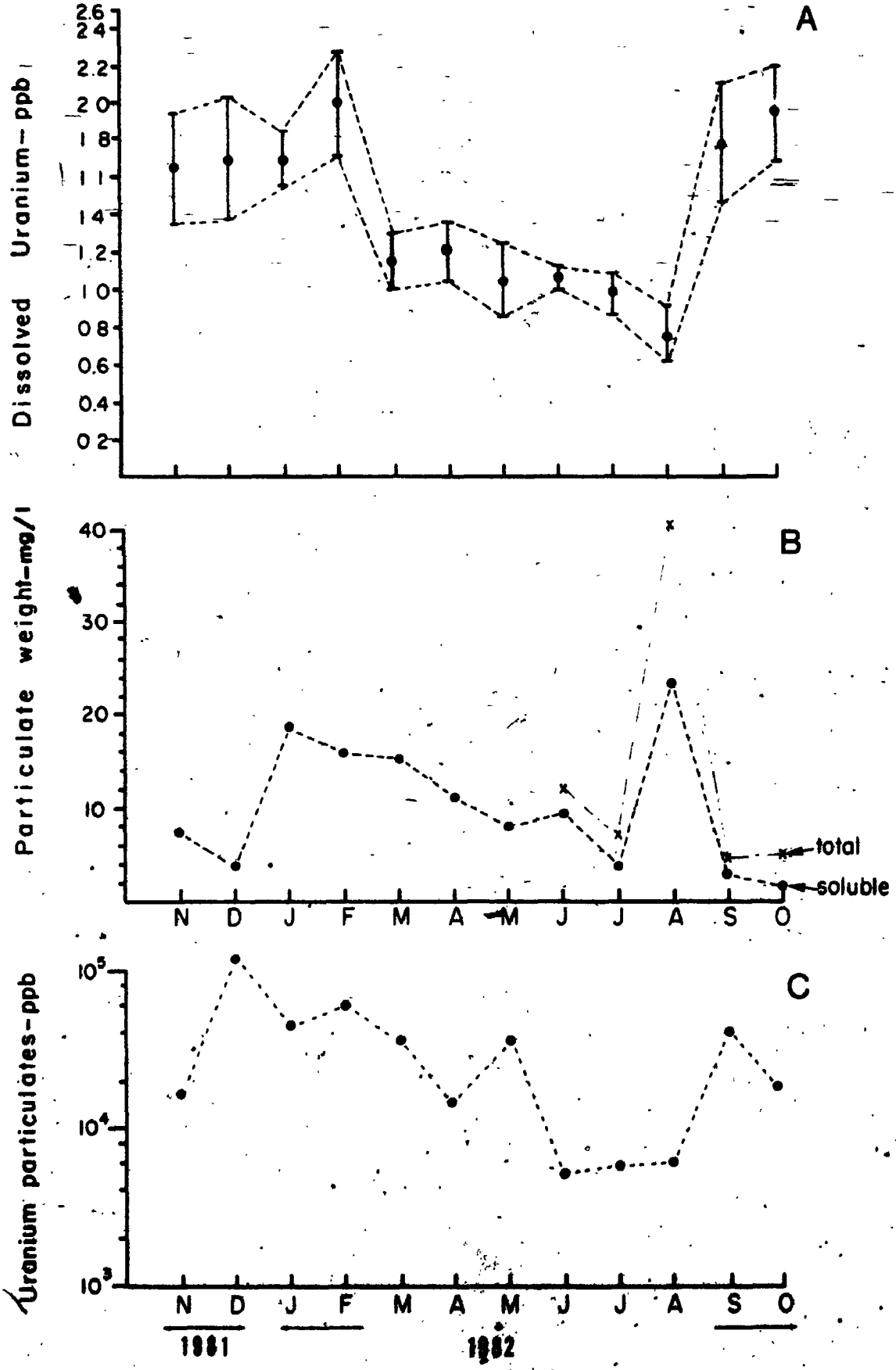
2 This column represents the average weight of >0.4 μm acid (HNO_3 + $HClO_4$) soluble particulates expressed in mg

3 This column represents the average total weight of >0.4 μm particules, expressed in mg

Figure 3.10 A. Variation of dissolved uranium in upper Thames River water as a function of time, averaged over all sampling stations (see Fig. 3.1, Table 3.1). Circles correspond to average, bars represent one standard deviation of the mean.

B. Variation of total, and acid (HNO_3, $HClO_4$) soluble particulate weight, expressed in mg per litre, in the upper Thames River, as a function of time, averaged over all sampling stations (see Fig. 3.1, Tables 3.2-3.13).

C. Variation of uranium in acid (HNO_3, $HClO_4$), soluble suspended particulates, as a function of time, averaged over all sampling stations (see Fig. 3.1, Tables 3.2-3.13).


A
Dissolved Uranium–ppb
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
B
Particulate weight–mg/l
40
30
20
10
total
soluble
N D J F M A M J J A S O
C
Uranium particulates–ppb
10^5
10^4
10^3
N D J F M A M J J A S O
1981
1982

over all sampling stations, are summarized in Table 3.14 and graphically portrayed as a function of time in Fig. 3.10.

The essential feature of these data is that the concentration of uranium in acid soluble suspended particulates is 10^3 to 10^5 times that of dissolved uranium in the host river waters. The average mass of acid soluble >0.45 μm suspended particulates, averaged over all sampling stations and all months, is 9.7 ± 6.6 1σ mg/litre, with a mean of 28,300 ppb U. Based on these results it is clear that this particulate fraction carries a significant proportion [9.7 mg/l x 28,300 ppb = 275 ng U/l] of the total riverine uranium flux, or about 15%, in relation to that transported directly in solution [10^3 g x 1.5 ppb = 1500 ng U/l] (see Fig. 3.11).

Solids soluble in acids are about a factor of ten larger by weight than the total suspended particulates, over the river system as a whole (Figs. 3.7, 3.8). The component in solution is remarkably uniform throughout the year, at about 500 ppm for all stations, whereas the suspended fraction fluctuates substantially, and varies markedly from station to station at a given time (Figs. 3.7, 3.8). A general correlation of total suspended particulates with discharge rate is evident for March plus April earlier in the year and November later in the year. The former peak occurs with higher discharge rates

accompanying a river in spate at thawing, whereas the latter appears to coincide with greater discharge during November rains for 1982 (Figs. 3.5, 3.6).

The August peak in suspended solids observed in this study (Fig. 3.10) is also present at five out of six stations graphed in Figs. 3.7 and 3.8. This peak is attributable largely to a pronounced increase in the acid soluble fraction, although a minor increment of acid insoluble particulates is also observed (Fig. 3.10). That the latter does not contribute significantly to the August peak of total suspended particulates is indirectly corroborated by noting that the mean monthly discharge rate at 4.5 m^3/sec is close to the annual low of 4.0 m^3/sec in June, 1982, when the insoluble suspended particulates are also low.

Microscopic examination of the >0.4 µm suspended particulate fraction revealed a high abundance of algae in August; in fact the August peak specifically coincides with the late summer algal bloom during this month. Particulates retained on the 0.4 µm filter papers were also analysed by X-ray diffraction from one location for each sampling month, both prior to and after the acid digestion procedure, for the purpose of identifying inorganic mineral particulates. Clays, quartz and feldspar are the dominant mineral species present at all times of the year. An inventory of the principal microorganisms is tabulated in

Table 3.15. Inventory of the principal suspended micro-organisms recovered from filtration of Thames River waters at specified sampling stations.

Sampling Time	Sampling Station 158.3	142.3	149.0(1)	114.8
1981 December	Oscillatoria	Oscillatoria	Oscillatoria	Oscillatoria
	-	Cladophora	-	-
	-	Scenedesmus	Scenedesmus	-
	-	Mougeotia	-	-
	-	-	Oedogonium	-
	Hydrodictyon	-	-	-
	Algal spores	Algal spores	Algal spores	Algal spores
	Diatoms	Diatoms	Diatoms	Diatoms
1982 January	Oscillatoria	-	Oscillatoria	Oscillatoria
	-	-	-	Chadophora
	-	-	-	Mougeotia
	-	-	-	Euglena
	Filamentus algae	Filamentus algae	Filamentus algae	Filamentus algae
	Algal spores	Algal spores	Algal spores	Algal spores
	Unicellular algae	-	-	-
	Diatoms	Diatoms	Diatoms	Diatoms
February	-	-	-	Spirogyra
	-	-	-	Oscillatoria
	Filamentus blue-green	Filamentus blue-green	Filamentus blue-green	Filamentus blue-green
	-	-	Filamentus green	-
	Algal spores	Algal spores	Algal spores	Algal spores
	Diatoms	Diatoms	Diatoms	Diatoms
March	Filamentus blue-green	Filamentus blue-green	-	Filamentus blue-green
	Algal spores	Algal spores	Algal spores	Algal spores
	Small unicellular	Small unicellular	Small unicellular	Small unicellular
	Diatoms	Diatoms	Diatoms	Diatoms
April	Scenedesmus	-	-	Scenedesmus
	Oscillatoria	-	Oscillatoria	Oscillatoria
	-	Euglenia	-	-
	Filamentus blue-green	Filamentus blue-green	Filamentus blue-green	Filamentus blue-green
	-	Algal spores	Algal spores	Algal spores
	Diatoms	Diatoms	Diatoms	Diatoms

Table 3.15 continued

Sampling Time	Sampling Station 158.3	142.3	149.0(1)	114.8
1982				
May	Scenedesmus	Scenedesmus	Scenedesmus	-
	Spirogyra	-	-	-
	-	Cosmarium	-	-
	-	Staurastrum	-	-
	Algal spores	Algal spores	Algal spores	Algal spores
	Filamentus blue-green	Filamentus blue-green	Filamentus blue-green	Filamentus blue-green
	Small unicellular	Small unicellular	Small unicellular	Small unicellular
	Diatoms	Diatoms	Diatoms	Diatoms
June	Scenedesmus	Scenedesmus	Scenedesmus	Scenedesmus
	-	Pediastrum	Pediastrum	-
	Closterium	-	Closterium	-
	-	-	-	Cosmarium
	-	Ulothrix	Ulothrix	-
	-	Chlorococcum	Chlorococcum	-
	-	-	Anacystis	-
	Filamentus blue-green	Filamentus blue-green	Filamentus blue-green	Filamentus blue-green
	Fragments of large colonials	Fragments of large colonials	-	-
	-	-	Small unicellular	-
	-	-	Euglena	-
	Diatoms	Diatoms	Diatoms	Diatoms
July	Scenedesmus	Scenedesmus	Scenedesmus	Scenedesmus
	Closterium	-	-	-
	-	Pediastrum	Pediastrum	Pediastrum
	-	-	Selenastrum	-
	Cladophora	-	-	-
	-	-	Crucigenia	Crucigenia
	-	-	-	Euglena
	-	-	-	Volvox
	-	-	-	Dinoclonium
	-	-	Anacystis	-
	-	Gloetrichia	Gloetrichia	-
	-	Ankistrodesmus	-	-
	-	Oocystis	-	-
	-	-	Cosmarium	-

Table 3.15 continued

Sampling Times	Sampling Stations 158.3	142.3	149.0(1)	114.8
1982				
July (cont.)	-	Gloeocapsa	-	-
	Filamentus blue-green	Filamentus blue-green	-	-
	Colonial motiles	Colonial motiles	Colonial motiles	Colonial motiles
	Diatoms	Diatoms	Diatoms	Diatoms
August	Scenedesmus	Scenedesmus	Scenedesmus	Scenedesmus
	Ulothrix	Ulothrix	Ulothrix	Ulothrix
	Pediastrum	Pediastrum	Pediastrum	Pediastrum
	Oedogonium	-	-	-
	-	Oscillatoria	Oscillatoria	Oscillatoria
	Stigeoclonium	-	-	-
	-	Closterium	-	-
	Spirogyra	-	-	-
	-	Crucigenia	Crucigenia	-
	-	-	Chorococum	-
	-	Oocystis	Oocystis	-
	-	-	Cladaphora	Cladaphora
	-	Netrium	-	-
	Filamentus green	-	-	Filamentus green
	-	Filamentus blue-green	Filamentus blue-green	-
	-	Small unicellular	Small unicellular	Small unicellular
	Diatoms	Diatoms	Diatoms	Diatoms
September	Scenedesmus	Scenedesmus	Scenedesmus	Scenedesmus
	Pediastrum	Pediastrum	Pediastrum	Pediastrum
	Closterium	Closterium	Closterium	Closterium
	Crucigenia	-	Crucigenia	Crucigenia
	-	Chloroccocus	-	Chloroccocus
	-	-	Coelastrum	-
	-	Anacystis	Anacystis	Anacystis
	Oocystis	-	Oocystis	-
	Oscillatoria	-	-	-
	-	-	Staurastrum	-
	Zygnema	-	-	-
	-	Ulothrix	-	-
	Small unicellular	Small unicellular	-	Small unicellular
	Filamentus green	-	-	-
	-	-	-	Filamentus blue-green
	Diatoms	Diatoms	Diatoms	Diatoms

Table 3.15 continued

Sampling Times	Sampling Stations			
	158.3	142.3	149.0(1)	114.8
1982 October	Scenedesmus	-	Scenedesmus	Scenedesmus
	Oscillatoria	-	Oscillatoria	Oscillatoria
	Crucigenia	-	Crucigenia	Crucigenia
	Closterium	-	Closterium	Closterium
	-	-	-	Ulothrix
	Small unicellular	-	Small unicellular	Small unicellular
	Filamentus green	-	Filamentus green	-
	-	-	-	Green colonials
	Algal spores	-	Algal spores	Algal spores
	Diatoms	-	Diatoms	Diatoms

Table 3.15 for each of the sampling months.

A minor peak in acid soluble particulate weight was also observed for January 1982. At this time of the year river waters were clear, and the discharge rates and total suspended particulates relatively low (Figs. 3.5-3.8). As for the August peak in acid soluble suspended particulates, microscopic examination revealed a relatively high proportion of algae.

In broad terms a correlation is present between dissolved uranium levels and the U content of acid soluble suspended particulates (believed to be largely microorganisms). For example both have a broad peak in December to February, followed by a continuous decline through August, with an upturn in September plus October (Fig. 3.10). By contrast a general antivariance is evident of weight versus the uranium content of acid soluble particulates (Fig. 3.10). The former parameter is depressed in December, peaking only in January and August, whereas the latter has a maxima at December 1981 and September 1982.

These data are collectively interpreted in terms of a relatively constant partitioning behaviour between dissolved uranium and that indigenous to microorganisms, such that variations in the former are tracked by the latter. The partition coefficient, K_d = U (microorganisms)/U (H_2O), is about 2×10^4. These results are commensurate with experimental data reported in chapter 2, where the

magnitude of uranium uptake by Ankistrodesmus depended on the level of the dissolved uranium supply. Whereas concentration factors, or uranium abundance in algae divided by that of the water refers to individual samples, the Kd defined above represents the partitioning of uranium between suspended microorganisms and their aquatic habitat, for the drainage system as a whole.

A pronounced bacterial maxima is present in August, as is the case for algal growth. Overall, the bacterial count varies erratically over the year, and from station to station. Minor peaks in bacterial count were recorded in May at 3 out of 6 stations, and in June at 3 out of 4 stations. A comprehensive examination of bacterial growth intensity through the seasons is difficult due to incomplete data (cf. Figs. 3.12, 3.13).

Both total and soluble riverine phosphate are relatively uniform through the year, irrespective of discharge rate, excepting a peak at 2 out of 5 sites in August (Fig. 3.14). Artificially raised soluble phosphate levels, chiefly from agricultural fertilizers and the detergent industry, are known to induce eutrophication of waters by inducing run-away algal growth. Phosphate in Thames river water at 0.1 to 0.6 ppm is about 1.5 to 10 times the world average river water phosphate concentration of 70 ppb (Holland, 1978). Given that the August phosphate peak of 0.6 ppm is present largely as a water insoluble

component, it cannot plausibly be invoked to account for the maxima in algae and bacteria at that time (Dillon, 1974, 1975). Rather, temperature effects have conventionally been quoted as the forcing factor in August algal and bacterial 'blooms'. This leaves open the question as to the cause of the August phosphate maxima. Higher river velocity, and thus suspended particulate load, cannot be involved in view of the low recorded discharge rate (Fig. 3.6). It is possible that the algal plus bacterial bloom is responsible for bioprecipitation of insoluble apatite [$(Ca_5(PO_4)_3(OH,F,Cl)$], but no compensating depression of soluble phosphate is observed. This leaves open the question as to the source of the August phosphate maxima.

3.5.1 The uranium complement of riverine particulates - problems of measurement

During the first seven months of collecting river waters it became apparent that a broad inverse relation existed between calculated uranium levels of acid soluble particulates and the measured soluble particulate mass. For instance, at 100 mg the average U content of acid soluble particulates is 3,000 ppb, whereas at 1 mg it is 40,000 ppb: i.e. the smallest masses apparently possessed the highest uranium abundance. This relation held for all months and sampling stations (Figs. 3.15, 3.16). Two interpretations of this trend are offered as follows:

Figure 3.12 Bacterial count, expressed in number per 100 ml over the interval November 1981 through December 1982, for specified sites (sample locations see Fig. 3.1). Source of data, Water Quality Monitoring Programme, Ontario Ministry of the Environment.

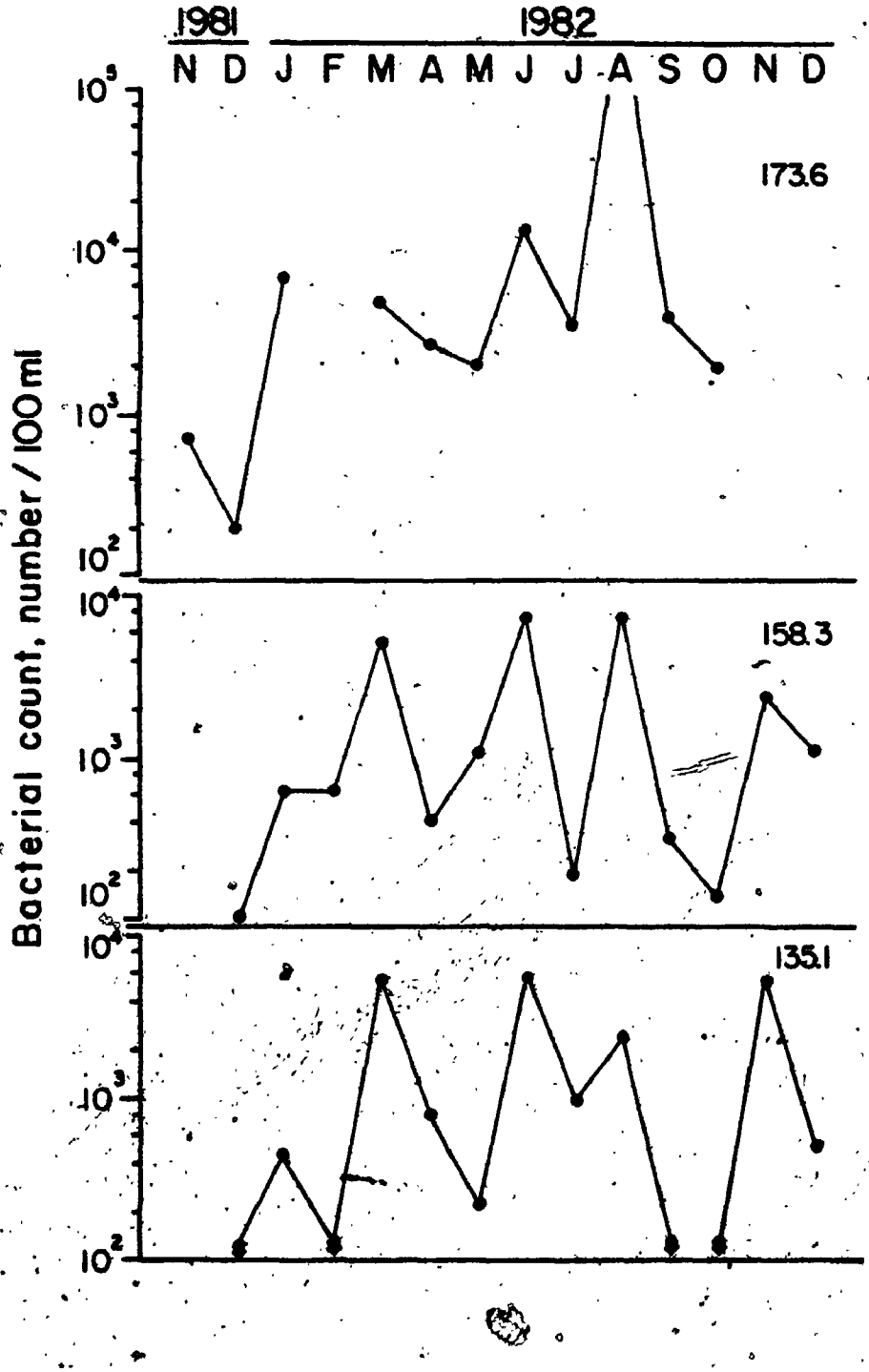

1981
1982
N D J F M A M J J A S O N D
Bacterial count, number / 100 ml
10^5
10^4
10^3
10^2
173.6
158.3
135.1

Figure 3.13 Bacterial count, expressed in number per 100 ml over the interval November 1981 through December 1982, for specified sites (sample locations illustrated in Fig. 3.1). Source of data - Water Quality Monitoring Programme, Ontario Ministry of the Environment. Original data incomplete as depicted.

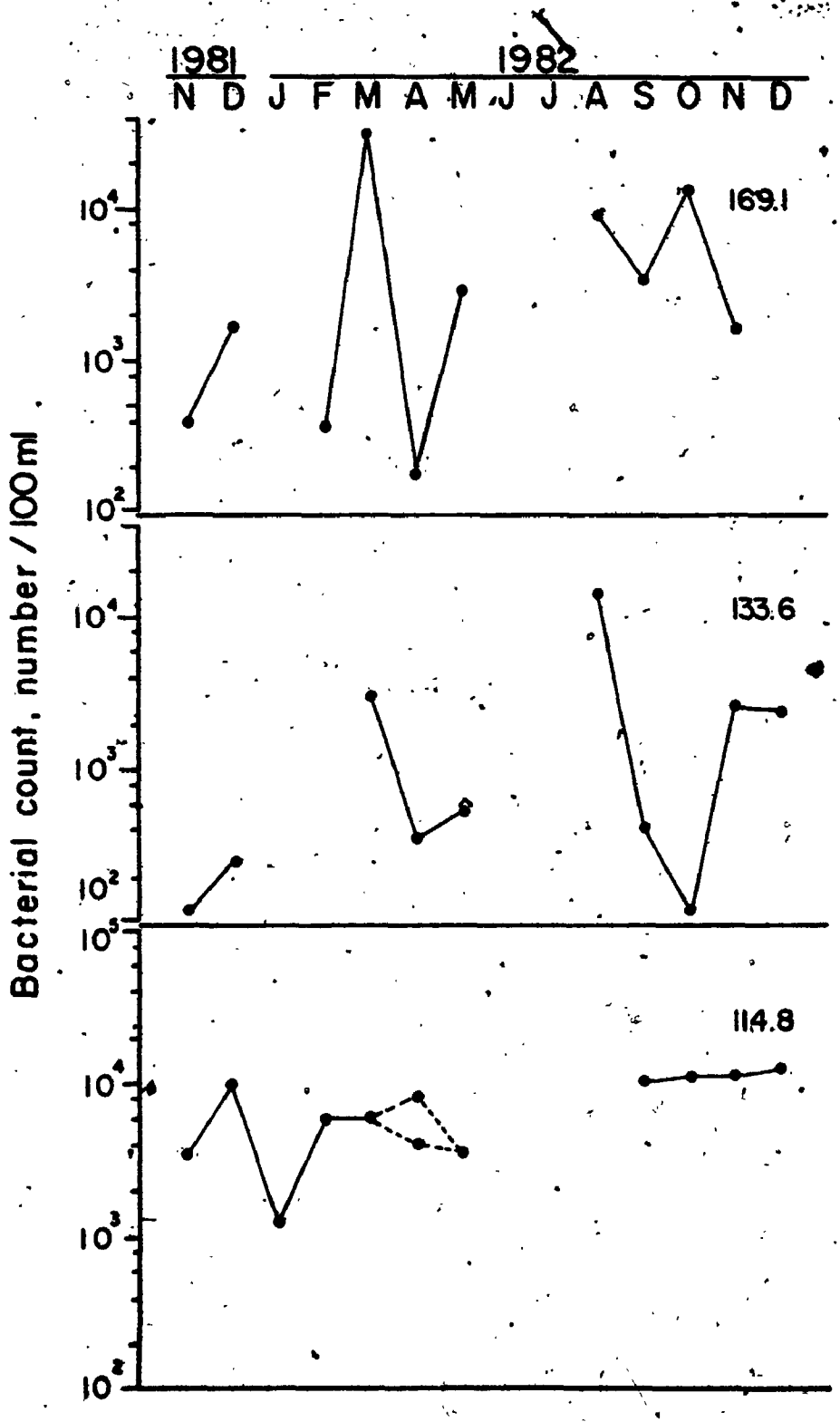

1981
1982
N D J F M A M J J A S O N D
Bacterial count, number / 100 ml
10^4
10^3
10^2
169.1
133.6
114.8

Fig. 3.14 Abundance of total and solute phosphate in Thames River waters at specified sites, for the interval November 1981 through October 1982. (For sample locations illustrated in Fig. 3.1.) Source of data - Water Quality Monitoring Programme, Ontario Ministry of the Environment.

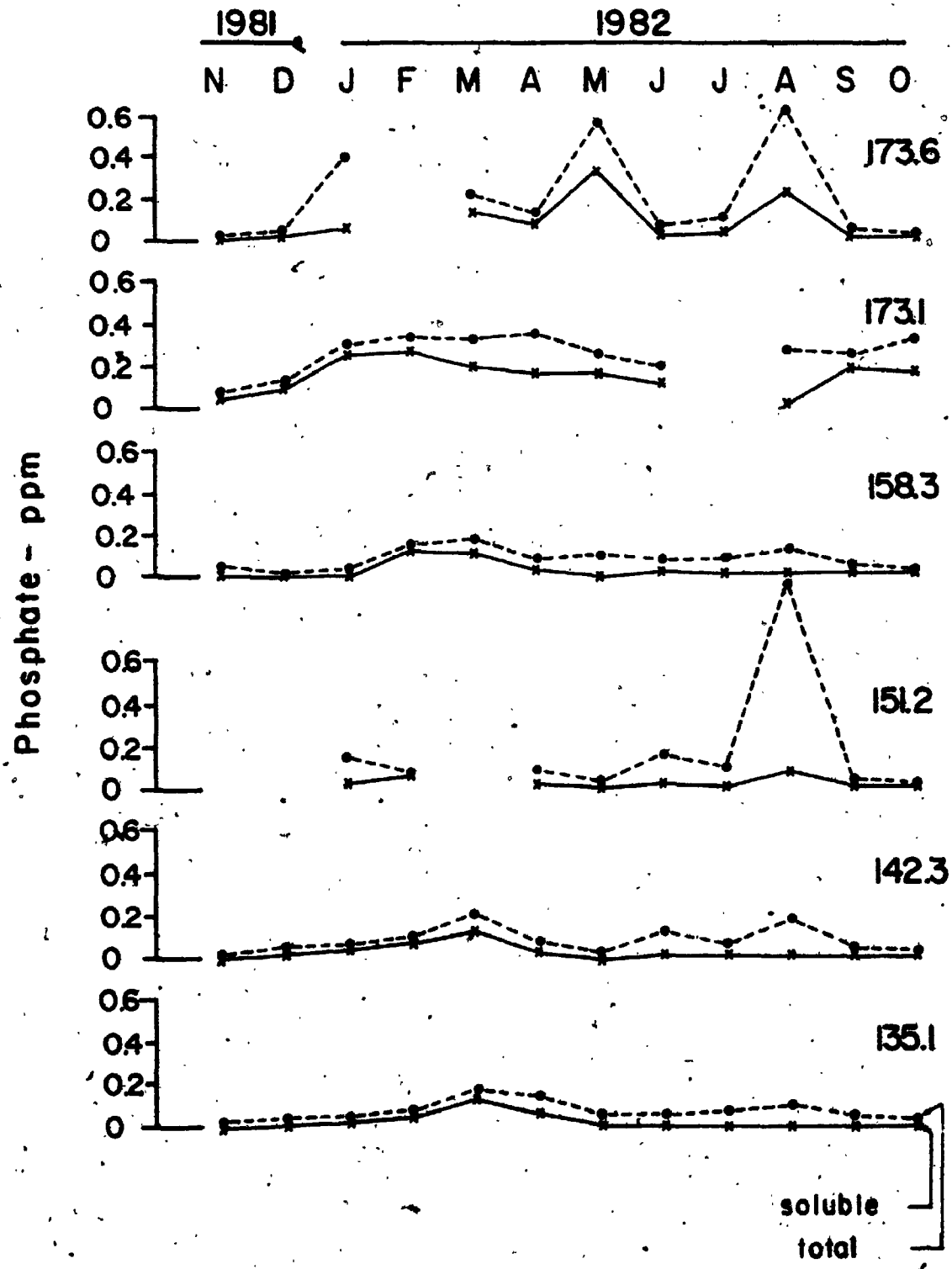

1981
1982
N D J F M A M J J A S O
0.6
0.4
0.2
0
173.6
173.1
158.3
151.2
142.3
135.1
Phosphate - ppm
soluble
total

Figure 3.15 Graph of uranium abundance versus weight of acid (HNO_3 + $HClO_4$) soluble suspended particulates for the Upper Thames River and its tributaries, plotted from the data in Tables 3.2-3.8. Logarithmic co-ordination.

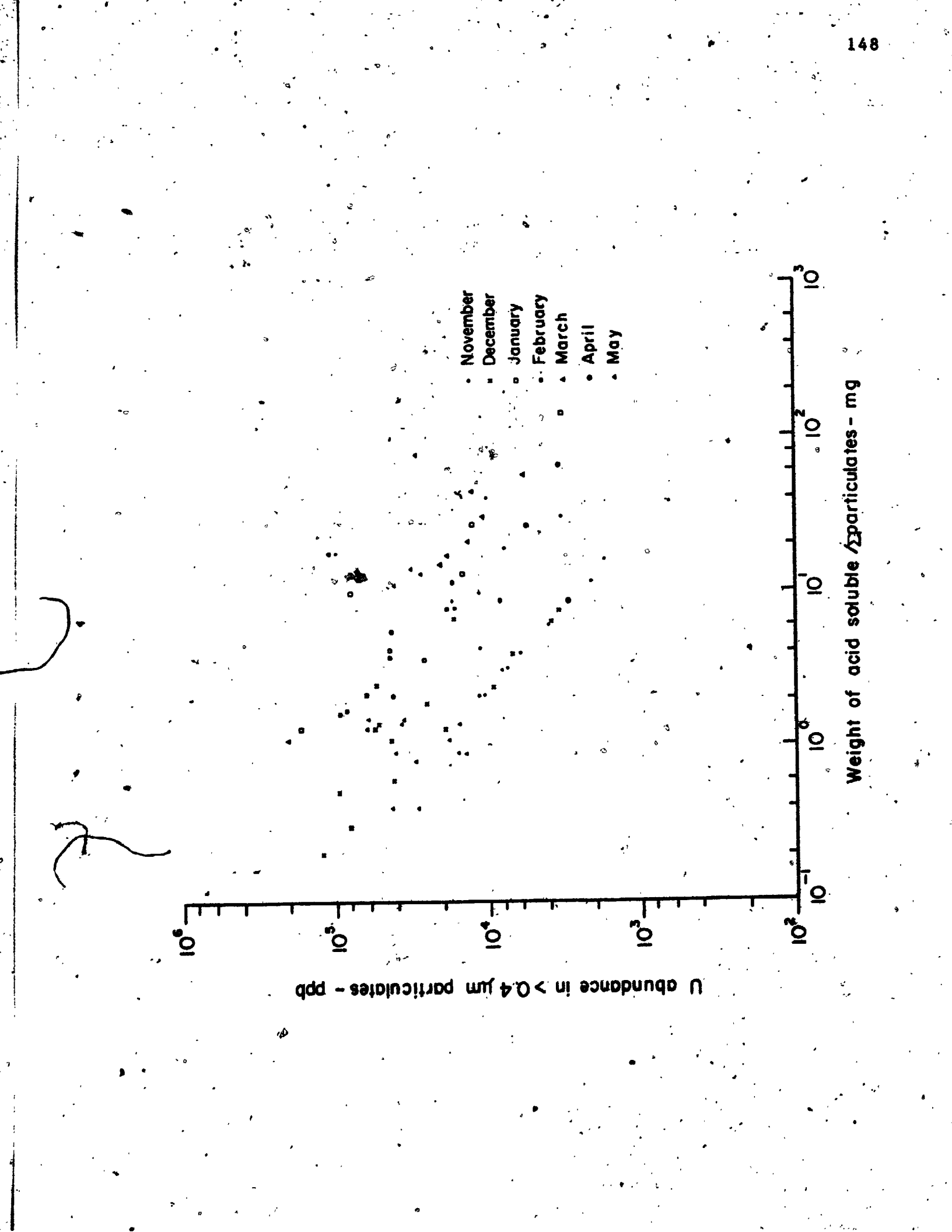

November
December
January
February
March
April
May
U abundance in >0.4 μm particulates - ppb
Weight of acid soluble Σparticulates - mg

Figure 3.16 Graph of uranium abundance versus weight of acid (HNO_3 + $HClO_4$) soluble suspended particulates for the upper Thames River and its tributaries, plotted from the data in Tables 3.9 through 3.13. Logarithmic co-ordinates.

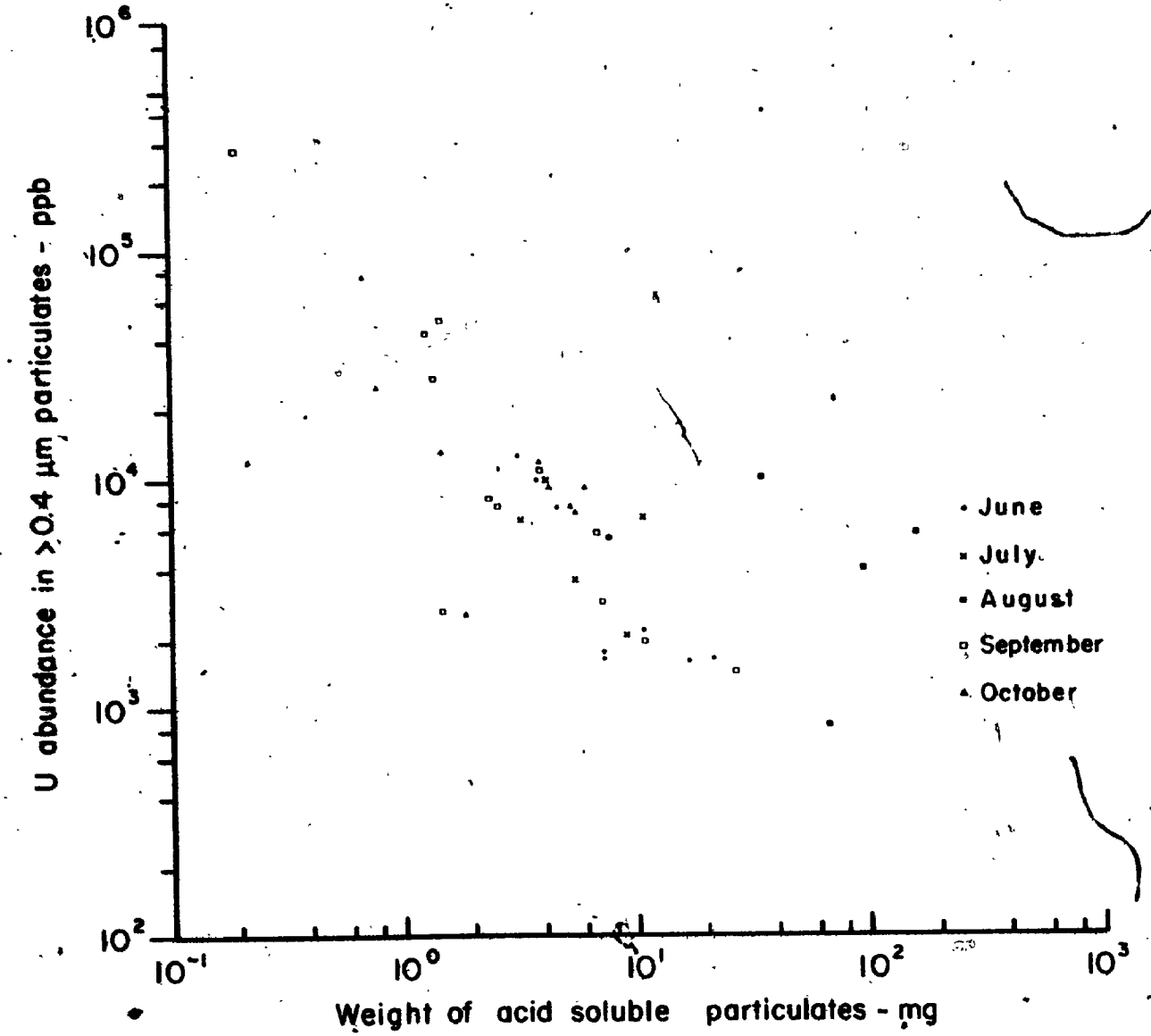
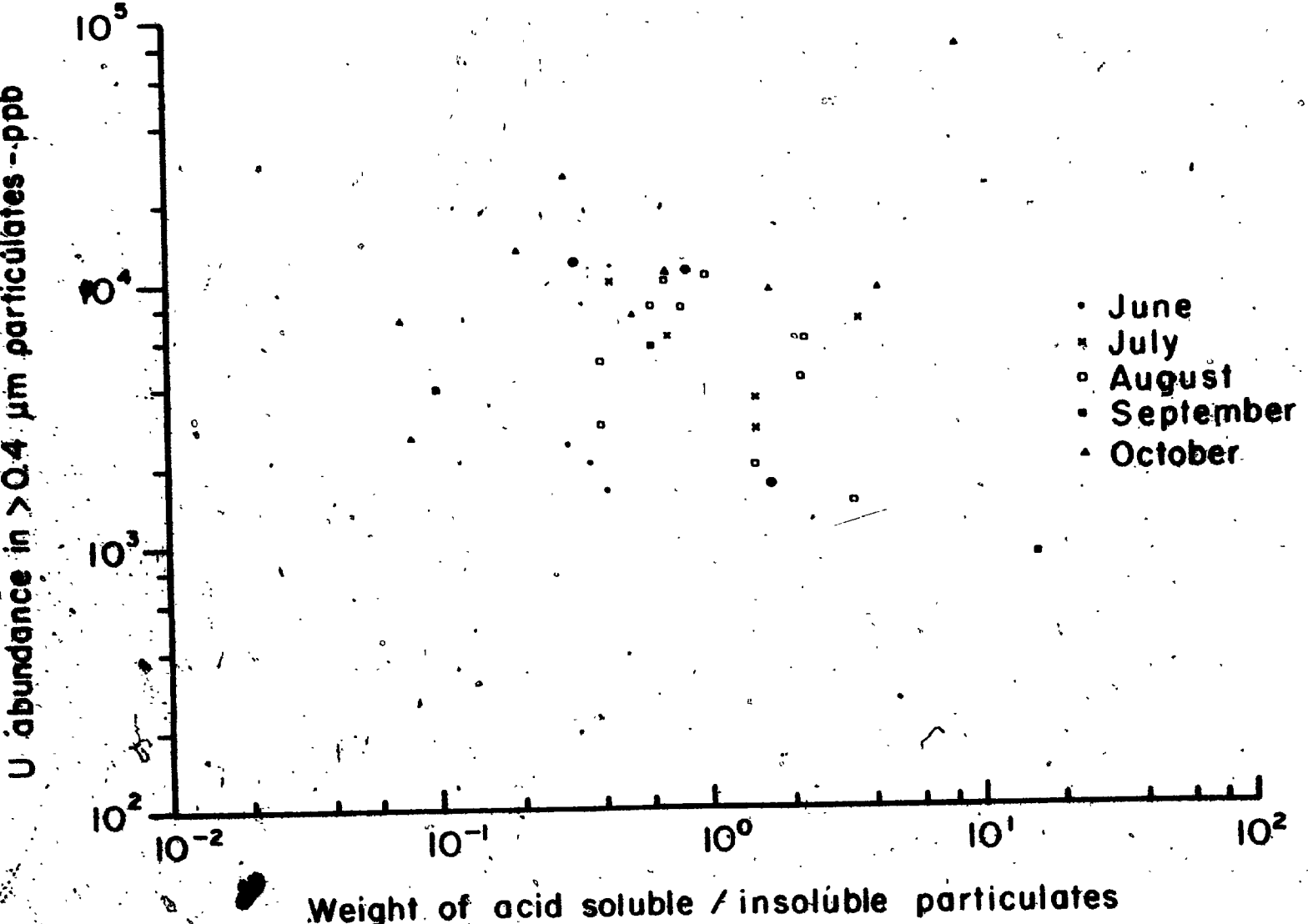
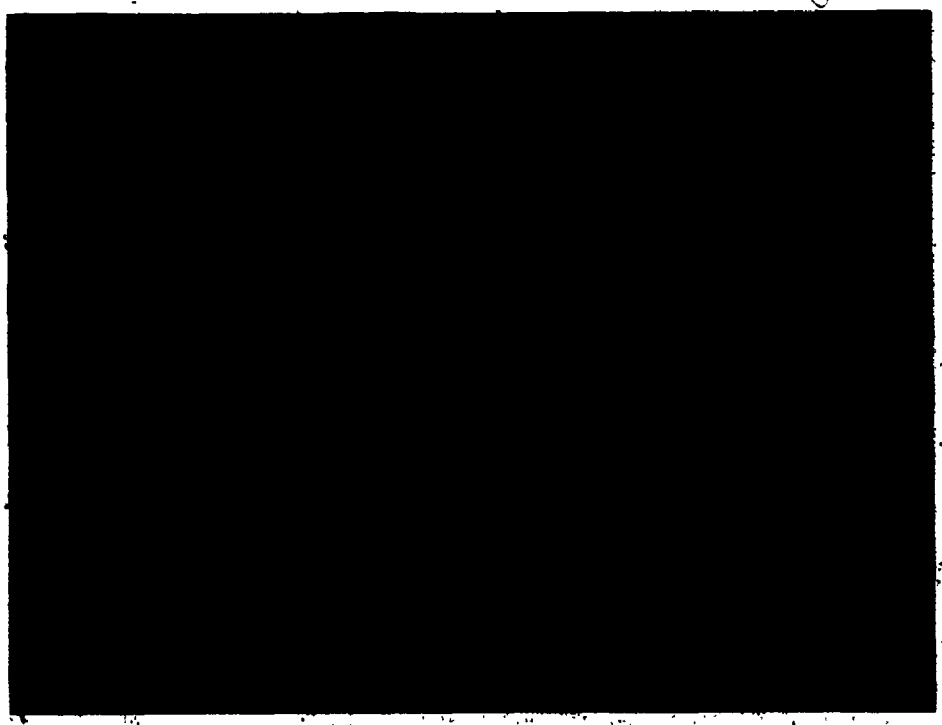
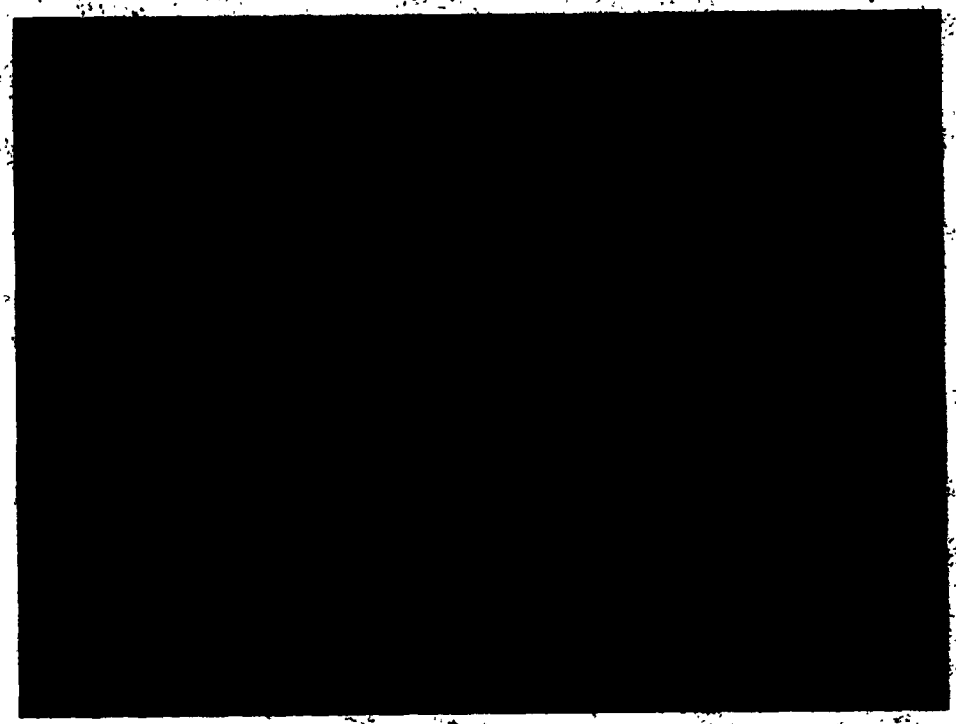

U abundance in >0.4 µm particulates - ppb
10^6
10^5
10^4
10^3
10^2
10^{-1}
10^0
10^1
10^2
10^3
Weight of acid soluble particulates - mg
June
July
August
September
October

Figure 3.17 Graph of uranium abundance in acid soluble suspended particulates versus the ratios of acid soluble to insoluble weights, for June through October 1982. Plotted from the data in Tables 3.9 through 3.13.


U abundance in >0.4 μm particulates - ppb
10^5
10^4
10^3
10^2
10^{-2}
10^{-1}
10^0
10^1
10^2
Weight of acid soluble / insoluble particulates
June
July
August
September
October





particulates and the acid soluble component. Scatter in plots of uranium abundance versus the ratio of acid soluble to insoluble fractions is so great that it is difficult to establish to which extent factor two discussed above influences high U levels at low particulate weights (Fig. 3.17).

Problems of this nature could be avoided in future studies using on site centrifugation to separate the micro-organisms from mineral particulates. However, in order to overcome the difficulties inherent in precise analysis of uranium in 0.1 mg of microorganisms collected from 2 litres, 10 litre samples would be required in future.

3.6 Intracellular crystals in filamentous algae

An unusually prolific bloom of filamentous algae was observed in the river Thames on the 17th August, 1982; this growth was sampled immediately. The algal bloom was located on the west bank, close to the University playing fields.

Microscopic examination of the filamentous algae, which was identified as a Spirogyra sp., revealed the presence of numerous intracellular crystals with a cross shape (Plates 3.2-3.3). Crystal-rich filaments were hand picked and then prepared for examination by SEM: these revealed details of the 'cross' shaped crystals, which typically had dimensions of 50 μm (Plates 3.4, 3.5).

The crystals were analysed for their chemical composition using an energy dispersive system (EDS) coupled to the SEM, by courtesy of Mr. Mike Powell. Only peaks of calcium (Ca) were recorded, apart from gold (Au) peaks arising from the sampling coating (Fig. 3.18). EDS analysis cannot resolve elements of light atomic number such as hydrogen (1 amu) through fluorine (17 amu). Thus the analysed crystals could plausibly be compounds of calcium with oxygen, carbon or flourine. Lowenstam (1981) has recorded the Ca-oxylate minerals whewellite and weddelite in organisms of the Kingdoms Protoctista, Fungi, Animalia and Plantea. Calci[illegible]oxylates or Ca-fluoride (cubic) are good candidates for the observed crystals. Whewellite is monoclinic whereas weddelite is tetragonal (Dana, 1932), and twinned crystal of the latter would fit well with the observed shapes. However, without further information on possible light atomic numbered elements associated with the calcium, their precise composition will remain unknown.

3.7 Summary and conclusions

The essential conclusions reached in this study of the upper Thames river and microorganisms of its aquatic environment are as follows:

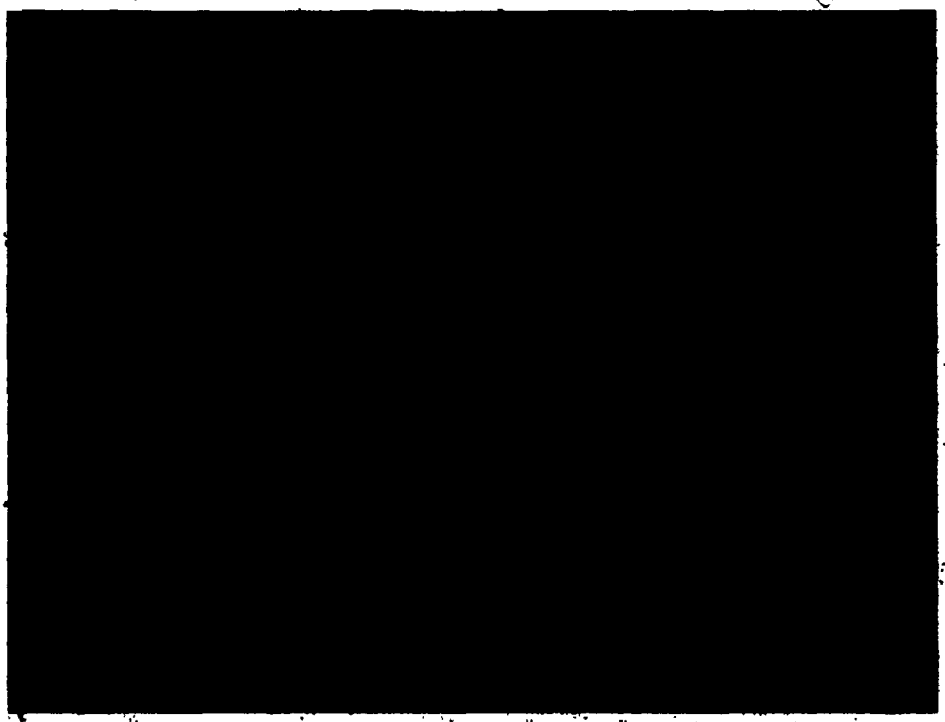
1. The average level of dissolved uranium is 1.46 ppb ± 0.61 1σ, representing about twice the global mean

riverine solute concentration of 0.6 ppb U.

2. Systematic seasonal variations of dissolved uranium abundance occur, peaking over the fall and winter months of September through February, and contrasting with depressed levels during the spring and summer months.
3. Winter and fall peaks correlate with higher discharge rates during thawing and rainfall maxima respectively.
4. Whereas variations in discharge rate of up to 100 fold occur over a year, excursions in aqueous uranium are at most a factor of two. Thus uranium supply to the river is more than compensated for by extra water in the river system.
5. Dissolved uranium rises sharply during thawing, correlating with increased suspended particulates and discharge rate. This uranium spike is probably indigenous to a transient meltwater surge.
6. The generally uniform levels of dissolved uranium throughout the year also holds for the total dissolved load which is relatively constant at ~300 ppm.
7. Suspended particulates, which represent about 10% of the total dissolved load by weight, show pronounced seasonal variations that correlate with riverine discharge rate.
8. Algae are abundant in Thames river waters at all seasons: they constitute a significant fraction (up to

Plate 3.2 Photomicrographs of Spirogyra sp. Upper view at low magnification, diameter of individual filaments approximately 500 µm.

Lower - detail of filaments illustrated above. Note intracellular crystals dispersed throughout filaments, especially in central portion of upper filament.



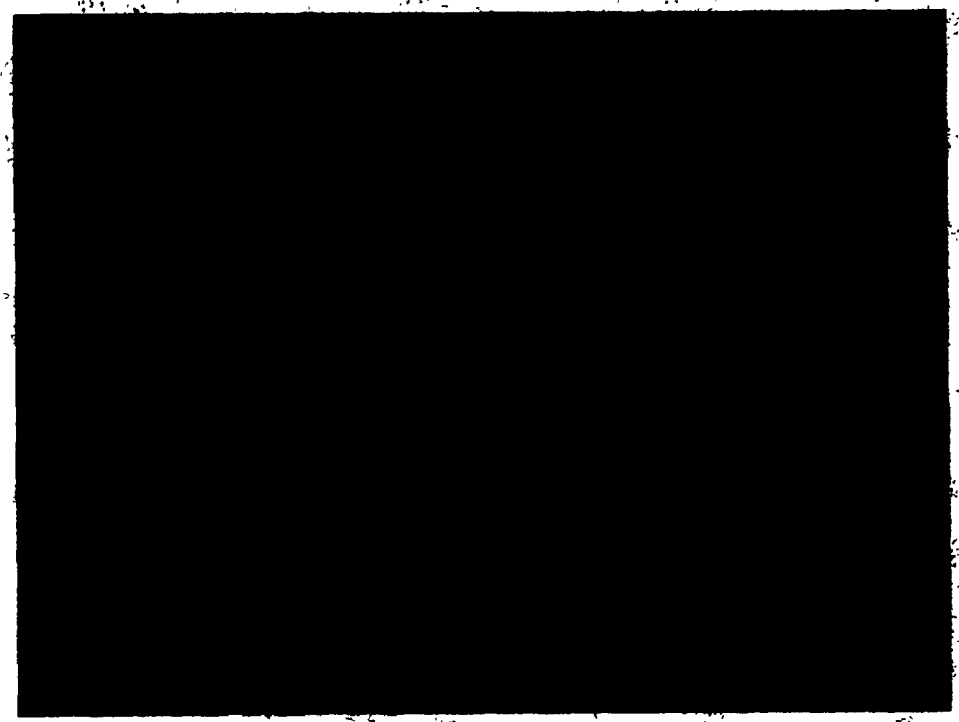


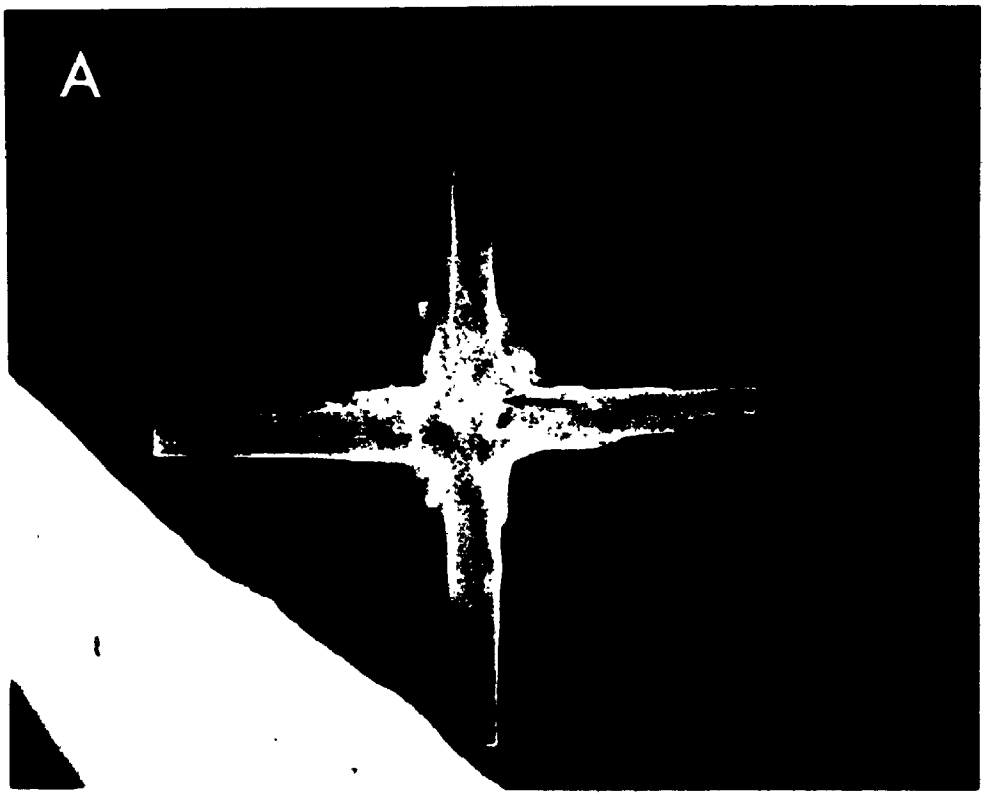
Plate 3.3 Photomicrographs of Spirogyra sp. containing intracellular crystals. Upper - filament diameter = 600 µm. Lower - filament diameter = 250 µm.

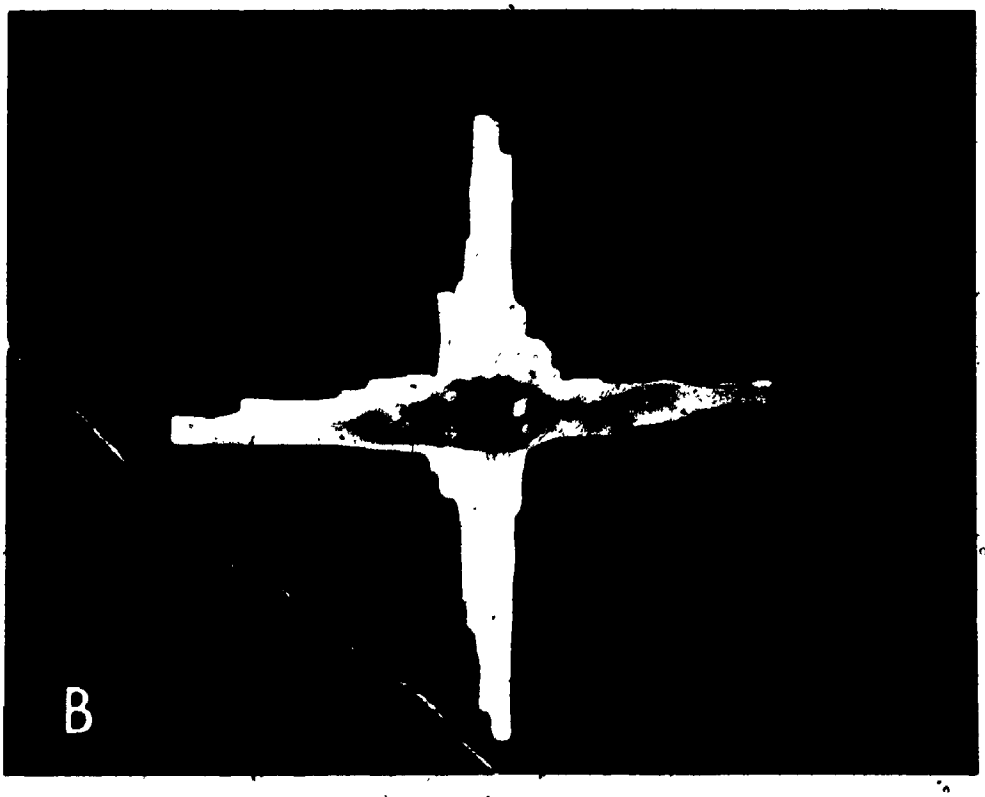
Plate 3.4 SEM micrographs of intracellular crystals within *Spirogyra* sp. A - horizontal field of view = 200 µm. B - horizontal field of view = 50 µm.


A


B

Plate 3.5 SEM micrographs of intracellular crystals within Spirogyra sp. A high, and B low contrast. Arrow points to 'seed' crystal. Horizontal field of view = 75 µm.

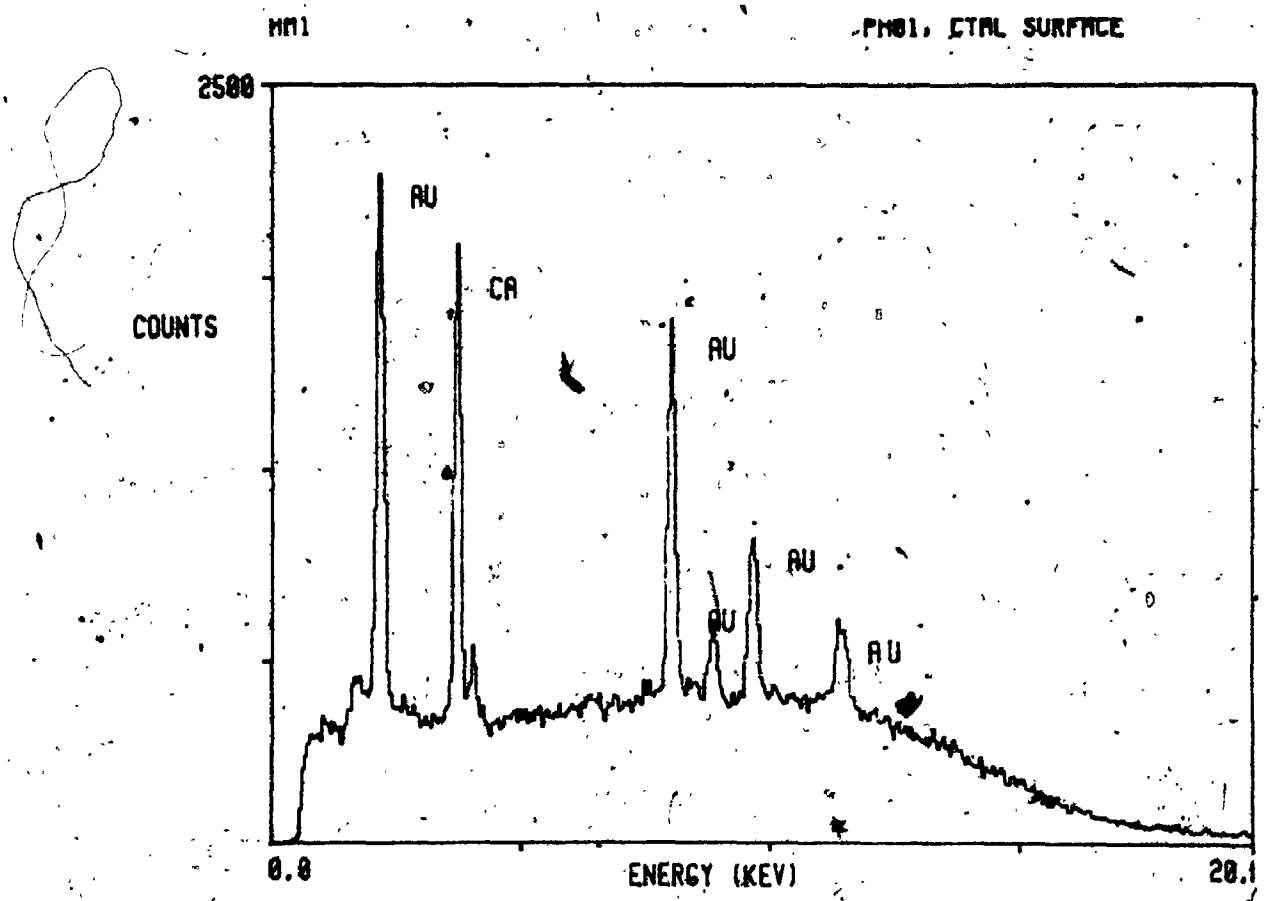

A


B

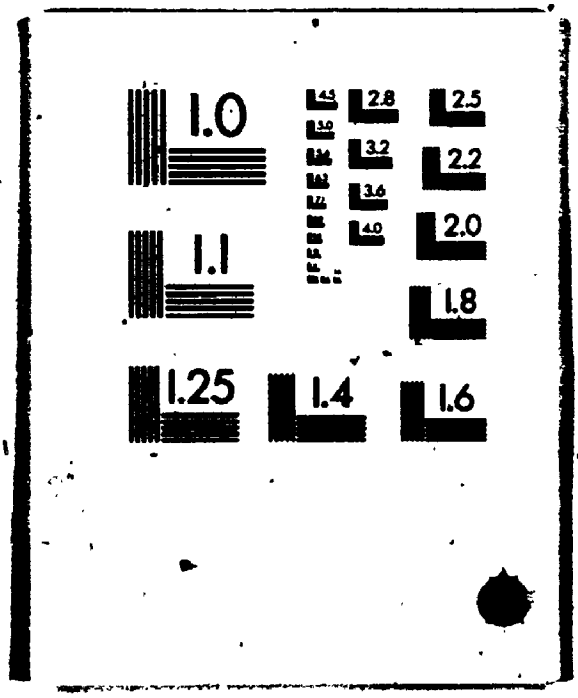
70%) of the total suspended particulates.

9. These algae have a uranium concentration 10^3 to 10^5 times that of their river habitat, and an average of 28,300 ppb U. Uranium concentrations of up to 0.75% by dry cell weight were recorded.
10. The average weight of the suspended acid soluble particulates (chiefly algae) is 9.7 $\pm$ 6.6 1σ mg/litre, and these microorganisms carry about 15% of the total riverine uranium flux. Peaks in algal growth were observed in January and August, the latter being a thermally induced bloom.
11. A broad correlation exists between levels of dissolved uranium in river water and the uranium concentration of suspended particulates (chiefly algae), implying a relatively constant partitioning of uranium between organisms and their water. The partition coefficient Kd is 2×10^4.
12. Intracellular crystals were found in one community of the filamentous algae Spirogyra sp. These are a calcium compound, combined with an unknown light atomic number element. The Ca-oxylates whewellite or weddelite are good candidates, but whewellite is monoclinic which makes it unlikely.
13. Phosphate levels in Thames river water, at 0.1 to 0.6 ppm, are about 1.5 to 10 times the world average river water phosphate concentration of 70 ppb.

Figure 3.18 Energy dispersive spectrum for microanalysis of intracellular crystals found within Spirogyra sp. Calcium (Ca) peaks arising from crystals, Au from the sample coating.


MM1
PM81, CTAL SURFACE
2500
COUNTS
AU
CA
AU
AU
AU
AU
0.0
ENERGY (KEV)
20.

3



runoff and localized groundwater seepage from the tailings impoundments (Cherry, 1980).

4.2.3 Physiography and site description

The Nordic Westarm and Nordic Main tailings impoundments (Fig. 4.1) are situated in a glaciated valley that runs east to west between bedrock uplands formed from Lower Proterozoic arenaceous sedimentary rocks that strike east-west and dip to the north. The rocks form a dip and scarp topography. The valley floor is underlain by Pleistocene sediments that comprise, from the original surface downward, sand and gravel of glaciofluvial origin over a layer of sandy, bouldery glacial till. A layer of black peat, 0.5 to 1 m thick, covers the sand and gravel in much of the valley area in which tailings were deposited. The peat formed in a spruce bog that formerly existed in this area.

Deposition of tailings in the Nordic Westarm impoundment began in 1957 and ceased in 1960. The impoundment was originally created by constructing dam A (see Moffett and Tellier, 1978), and discharging the tailings so that the clear effluent flowed into Westner Lake (Fig. 4.1). Subsequently, Dam B was constructed and the flow was diverted to discharge through the east end. The tailings were deposited in the middle of Westarm, and as time progressed the discharge point was moved westward towards dam B (Moffett and Tellier, 1978). In order to obtain more storage

specific objectives may be listed as follows:

1. To establish the aqueous uranium concentration in waters of the Elliot Lake region upstream, in the vicinity of, and downstream of tailings impoundments.
2. To determine if any seasonal variation occurs in dissolved uranium within these three specific domains of the drainage basin.
3. To measure the uranium content of suspended particulates, especially algae, and communities of filamentous algae.
4. To compare uranium levels in Elliot Lake waters and algae with those found for the experiments reported in chapter 2.

This chapter continues with a concise background of the history of mining and tailings accumulations at Elliot Lake, including the disposition of tailings impoundments, a summary of hydrological processes, water treatment, and other factors that are germane to the present study. The timing, locations and details of sampling are presented, followed by a report of data collected. Results are then discussed in the context of the specific objectives outlined above, and compared to other studies.

4.2 Elliot Lake: Introduction

4.2.1 Location

The Elliot Lake district is located in Ontario,

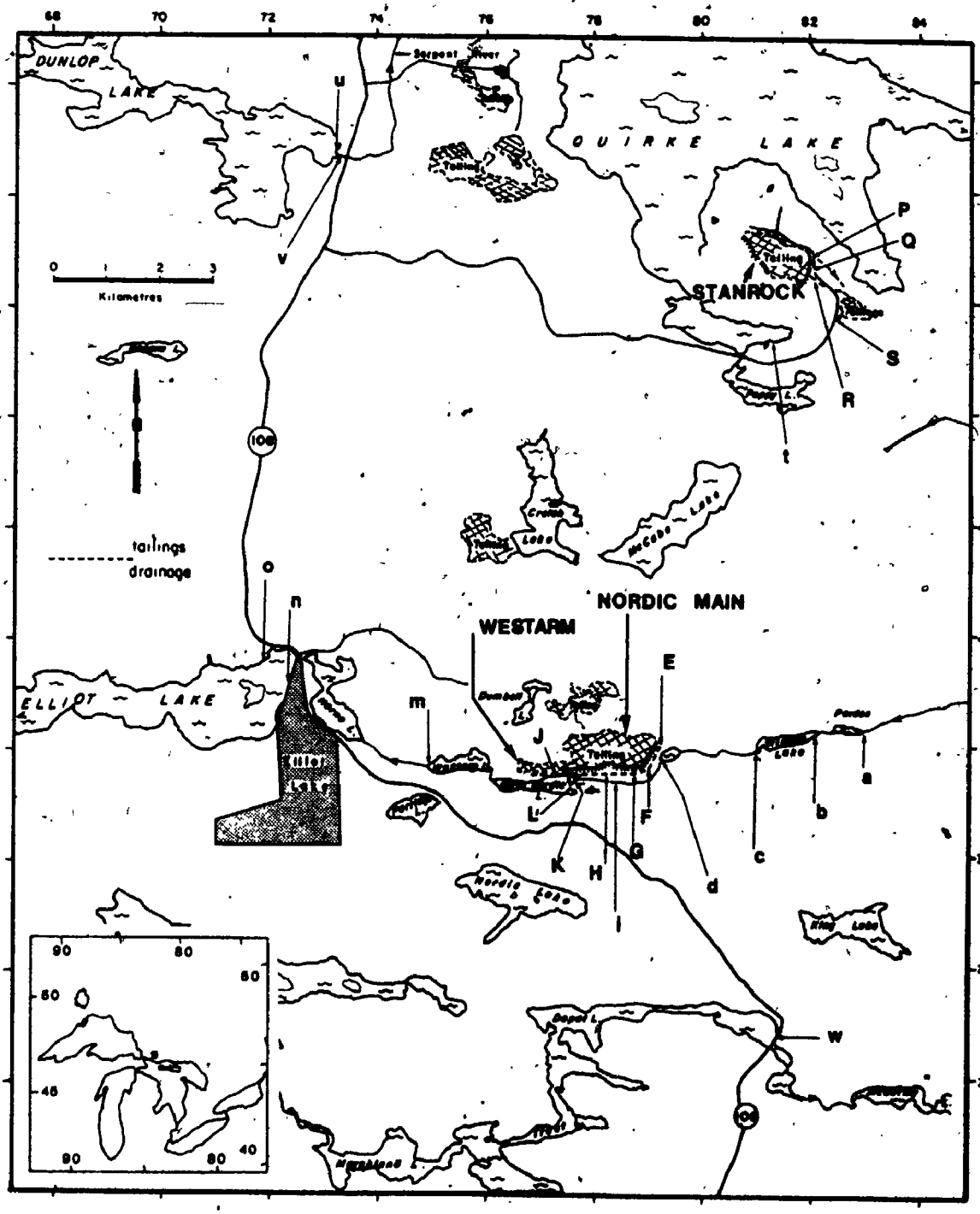
Canada, approximately 40 km north of Lake Huron's north shore and 130 km due east of Sudbury (Fig. 4.1). The region has a temperate climate with an annual precipitation of 800 mm: precipitation is fairly evenly distributed throughout the year. From November to late April, or even May the area is snow covered. Summer temperatures vary from 10°C to 30°C.

4.2.2 History of uranium mining

Uranium mining and milling started in the Elliot Lake area in 1954. Since then a total of 12 mines and 10 mills have been in operation, producing approximately 100 million tonnes of tailings. There are currently 3 mills operating at Elliot Lake.

The uranium ore is milled and extracted using a sulfuric acid treatment which is called a leach process. The sand-size and silt-size tailings along with process water from the milling operations are discharged hydraulically into surface impoundments as a slurry consisting of 30 to 40 percent solids. The acid process water from the mill is currently neutralized with lime to a pH of 9-11 before discharge to the tailings impoundments. In the 1950's and 1960's the process water was only neutralized to a pH of 6-7. The tailings and process water from the mills at Elliot Lake have been deposited in a variety of impoundments, most of which are presently

Figure 4.1 Map of the Elliot Lake district, illustrating the disposition of the principal tailings impoundments, along with sampling stations for natural and tailing waters. Natural waters lower case alphabet, tailings waters in capitals. Co-ordinates from Map Elliot Lake 41 J/7 Edition 2, 1975.


DUNLOP LAKE
QUIRKE LAKE
STANROCK
WESTARM
NORDIC MAIN
ELLIOT LAKE
Serpent River
Kilometres
tailings
drainage
108
P
Q
S
R
t
U
V
o
n
m
E
J
L
F
G
K
H
I
a
b
c
d
W

inactive (Feenstra et al., 1981).

Suspended solids in slurry discharged to the tailings impoundments contain 3 to 7 percent pyrite (FeS_2). When the discharge of tailings and neutralized process water ceases and the tailings are exposed to the atmosphere, pyrite oxidation can occur close to the surface (generally within one metre). Pyrite oxidation, according to the following reaction:

$$2FeS_2+7O_2+2H_2O \rightarrow 2Fe^{2+}+4SO_4^{2-}+4H^+$$

produces low pH conditions with accordingly high concentrations of iron and sulfate in the tailing porewater. The low pH conditions and high concentrations of iron plus sulfate also result in the release of abundant dissolved heavy metals together with the radionuclides U, Th, Ra and other daughter elements from the tailings solids, into tailings porewater.

This porewater could move out of the tailings impoundments either via surface water runoff or by groundwater seepage, and the toxic species could collectively have a potentially unacceptable effect on the surrounding environment (Cherry, 1980). At the present time in the Elliot Lake area, the effect of the tailings impoundments on the surrounding environment is partially controlled by the collection and treatment of contaminated surface water

runoff and localized groundwater seepage from the tailings impoundments (Cherry, 1980).

4.2.3 Physiography and site description

The Nordic Westarm and Nordic Main tailings impoundments (Fig. 4.1) are situated in a glaciated valley that runs east to west between bedrock uplands formed from Lower Proterozoic arenaceous sedimentary rocks that strike east-west and dip to the north. The rocks form a dip and scarp topography. The valley floor is underlain by Pleistocene sediments that comprise, from the original surface downward, sand and gravel of glaciofluvial origin over a layer of sandy, bouldery glacial till. A layer of black peat, 0.5 to 1 m thick, covers the sand and gravel in much of the valley area in which tailings were deposited. The peat formed in a spruce bog that formerly existed in this area.

Deposition of tailings in the Nordic Westarm impoundment began in 1957 and ceased in 1960. The impoundment was originally created by constructing dam A (see Moffett and Tellier, 1978), and discharging the tailings so that the clear effluent flowed into Westner Lake (Fig. 4.1). Subsequently, Dam B was constructed and the flow was diverted to discharge through the east end. The tailings were deposited in the middle of Westarm, and as time progressed the discharge point was moved westward towards dam B (Moffett and Tellier, 1978). In order to obtain more storage

volume, dam C was raised in 1960 to create the Nordic Main impoundment and the Westarm tailings area was left inactive (Cherry et al., 1980).

Tailings were deposited in the Nordic Main area until 1968 when the Nordic mine and mill closed operations. Deposition originally began in the southwest corner, subsequently extending northward and eastward. The tailings deposit was formed of broad low-angle alluvial fans in which the coarser tailings were deposited nearest to the discharge pipe and the finest particles settled farthest away. After closure of the mill in 1968, the eastern portion of the Nordic Main area contained ponded water on the tailings. This water was drained off in 1970-71 (Cherry et al., 1980).

A program for covering the tailings with grass started in 1973. By 1978 much of the area was covered, with the exception of areas having a high water table and fine-grained tailings, where surface treatment, fertilization, and seeding has not produced growth. In 1978 the surface of the Nordic Main tailings was treated employing a thin layer of crushed lime, fertilizer and grass seeds. By June 1978 and 1980 dense grass cover was established over the entire surface of the tailings (Murray and Moffett, 1977). Stabilization of the tailings by establishing a grass cover has greatly reduced dispersal of fine tailings particles by wind, minimized the effects of sheet erosion, and has

probably reduced to some extent the amount of water that annually infiltrates to the water-table zone in the tailings by increased runoff and evapotranspiration. An additional objective of the program for establishment of vegetative cover on the tailings is to reduce the rate of pyrite oxidation by rain or melt-water incursion (Garber and Ibbotson, 1979). In 1982 wild life was observed on the tailings, and the Elliot Lake rifle club established practices on this tailings area.

The potential for adverse effects on the ecology of streams is related both to the acid conditions and low-pH or linked high concentrations of toxic metals in the tailing seepage waters.

4.2.4 Stanrock tailings area

The Stanrock tailings area is a tailings impoundment located 0.8 km east of the Denison Mines Stanrock mill and 1.0 km south of the Can-Met mill. This tailings management area covers 83 ha and contains approximately 5.5 million tonnes of tailings. The tailings in the east-central portion of the impoundment are up to 20.9 m in thickness. Tailings from the Can-Met mill were discharged to the impoundment beginning in 1957, continuing until 1960. Tailings from the Stanrock mill were discharged to the impoundment beginning in 1958, and through to 1964. The Stanrock tailings area was purchased by Denison Mines in

1973.

The tailings impoundment is enclosed by bedrock ridges to the north and southeast, by dam A to the east and dam, B, C and D to the southwest (Fig. 4.1). The bedrock ridges consist of relatively low permeability metamorphosed conglomerates, greywackes and quartzites of the Gowganda Formation. Dam A is approximately 18.3 m high, consisting of cycloned sandy tailings from the Can-Met mill. Dams B, C and D range from 6.1 m to 12.2 m in height, consisting of spigotted tailings from the Stanrock mill. The tailings are underlain by thin, discontinuous deposits of sand and gravel with a depth of 4.8 m, fine sand, silt and peat.

Tailings from the Can-Met mill were discharged to the tailings from the north side of the impoundment area, whereas tailings from the Stanrock mill were discharged from the west end of the impoundment. Coarser grained sandy tailings were therefore deposited at the north side and west end, while finer grained silty tailings were deposited towards the east end of the impoundment. The surface of the tailings slopes from west to east at a gradient of approximately 0.5%. Precipitation and catchment runoff collects in a small headpond adjacent to dam A at the east end of the impoundment before discharge through a rock lined spillway at dam A constructed in 1979 (Feenstra et al., 1981).

4.3 Sampling and analytical methods

The spring and fall seasons were chosen for collecting samples from the Elliot Lake mining area, the reason being that these are the times when maximum algal growth typically occurs. In 1981 sampling was performed from 29 August to 1 September; for 1982, 5-10 May in the spring and 28 to 30 September for the fall collection. At each sampling station 2 litres of water was collected by submerging two 1 litre nalgene plastic bottles into the water. Each bottle was prewashed with HNO_3 and rinsed well with DIW. After each sample was collected, 10 ml of HNO_3 was added and the bottles stored until arrival at the University of Western Ontario. Where thick carpets of unicellular algal colonies occurred afixed to the stream or tailings dralnage channel bottom, the algae were collected by plastic spatula and stored in plastic bags, or in plastic bottles.

Filamentous algae were washed on site with water, and the excess water drained off. At UWO the algae were dried to constant weight and then digested (see Appendix I). Alternatively, algae were frozen if digestion was not possible right away. Digestion of algae used equal amounts of nitric acid and perchloric acids, in a teflon beaker on a hot plate at 70°C. The solution was transfered to an appropriate volumetric flask, the beaker was washed twice with 50% HNO_3, once with DIW, and then taken to volume with DIW (see Appendix I). In some instances this volume was

Table 4.1 Uranium abundance in river, lake and tailings water, vicinity of Elliot Lake (U in ppb).

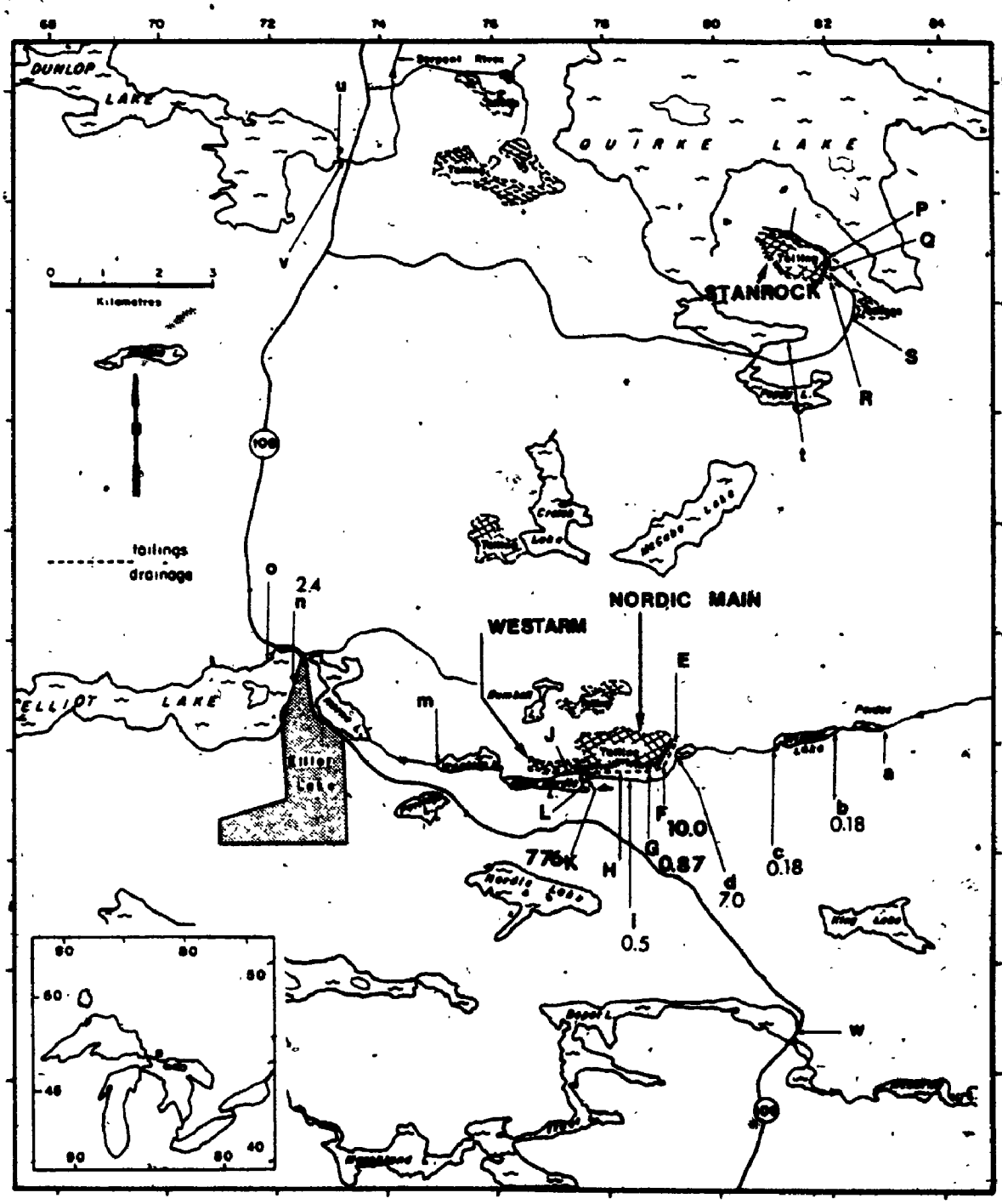
Sampling Station	Geographical Location	Coordinates†	Sept. 1981	May 1982	Sept. 1982
Natural drainage above Nordic tailings					
A	Pardee Lake, E. end	829, 384		0.10 [0.20]	0.40
B	Stintson Lake, E. end	819, 382	0.18	0.20 [0.05]	0.05
C	Stintson Lake, W. end	810, 379	0.18(0.18)	0.65 [0.41]	0.05
D	Lake 200 m to E. of tailings	791, 378	7.0	0.49 [0.06]	0.05
Tailings drainage - Rio Algom, Nordic-Westarm					
E	N. drainage of tailings dam	792, 381		59 [67]	165
F	Drainage ditch below (E)	790, 377	10.0(7.6)	51 [66]	153
G	Drainage ditch below (F)	789, 375	0.87(0.87)	56	153
H	Drainage ditch below (F)	786, 375		58	148
I	Natural drainage to E. of tailings ditch	793, 378	0.5(0.5)		0.2
J	Drainage ditch to W. end of tailings	774, 375	128, 207	63 [66]	
K	Drainage ditch to W. end of tailings	776, 376	776, 376	115 [113]	
L	North Nordic Lake - E. end	775, 375		29 [31]	
M	Westner Lake, W. end	750, 376		5 [9]	3.1
N	Elliot Lake, E. end (park)	725, 395	2.4(1.0)	3	
O	Elliot Lake, E. end (jetty)	720, 396			9.4
Natural drainage above Quirke tailings					
U	Serpent River - E. end Dunlop Lake	1733, 487			0.2
V	Serpent River - downstrem of Dunlop Lake	734, 487			0.2
W	Depot Lake, E. end (by airport) (Marshland River)	812, 326		1.5 [1.6]	2.7
Tailings drainage - Denison Mine Stanrock					
P	Quirke tailings, N.E. end	819, 470		80	177
Q	Tailings drainage S.E. end	820, 466		93	330
R	Tailings drainage S.E. end	823, 464		297 [302]	661
S	Tailings dam H_2O	826, 459			16.5
T	Quirke Lake	813, 454		<0.2	15.3
Natural drainage downstream of Elliot Lake					
X	Serpent River on Sudbury Sault St. Marie Road			1.9 [3.2]	1.2
Y	French River water			0.56 [0.16]	
Z	Lake Huron Water - Lion's Head				0.6
ZZ	Lake Huron Water - Red Bay				0.6

† Map Elliot Lake 41 J/7 Edition 2, 1975

figures in round parentheses are duplicate analyses by fluorimetry

Figures in square brackets are analyses by DNC

Figure 4.2 Dissolved uranium content of river, lake and tailings waters of the Elliot Lake district, September 1981. Light numerals represent data for natural waters, bold face for tailings waters. U in ppb.


DUNLOP LAKE
QUIRKE LAKE
ELLIOT LAKE
STANROCK
NORDIC MAIN
WESTARM
Kilometres
tailings
drainage
P
Q
S
R
t
V
U
o
n
2.4
m
E
J
L
F
G
H
I
K
10.0
0.87
0.5
776
7.0
a
b
0.18
c
0.18
d
W
108

divided for analysis of uranium by both fluorimetry and delayed neutron counting.

Water was filtered through Millipore filter paper size RA-0.45 µm, or RA-1.2 µm followed by RA-0.45 µm, depending on the size and quantities of particles in water samples. Waters were then evaporated in teflon beakers on a hot plate at 70°C, transferred to an appropriate volumetric flask and finally taken to volume with DIW. The analyate was then divided into two aliquots, if appropriate, for analysis of uranium by fluorimetry and delayed neutron counting.

Particulates retained on millipore filter papers from filtration of the two litre water volumes were digested and analysed for uranium as described in chapter 2 and Appendix I.

4.4 Dissolved uranium in waters of the Elliot Lake region.

4.4.1 Natural drainage upstream of tailings.

The uranium solute concentration for river, lake and tailings waters of the Elliot Lake region, at three specified sampling times, is reported in Table 4.1, and mapped out in Figs. 4.2-4.4. Upstream of the Nordic tailings, natural drainage waters of Pardee and Stintson Lakes along with their interconnecting streams average 0.18 ppb, 0.36 ppb $\pm$ (0.25 1σ), and 0.14 ppb ($\pm$ 0.17 1σ) for the three sampling periods, respectively (Fig. 4.2-4.4). The first

Figure 4.3 Dissolved uranium content of river, lake and tailings waters of the Elliot Lake district, May 1982. Light numerals represent data for natural waters, bold face for tailings waters. U in ppb.

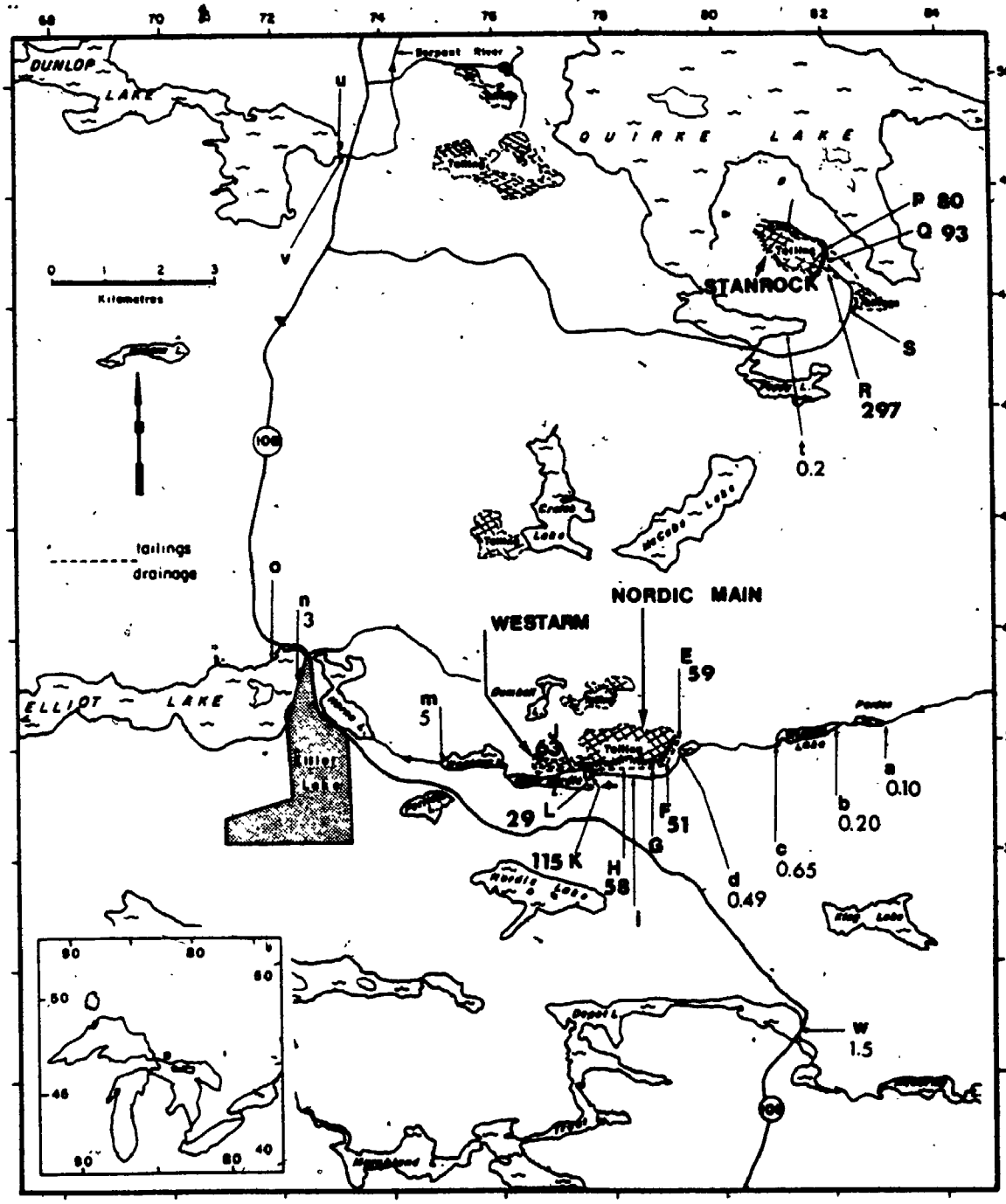

DUNLOP LAKE
QUIRKE LAKE
ELLIOT LAKE
STANROCK
P 80
Q 93
S
R
297
0.2
NORDIC MAIN
WESTARM
E
59
F
51
G
H
58
I
L
29
115 K
a
0.10
b
0.20
c
0.65
d
0.49
W
1.5
m
5
n
3
o
tailings
drainage
Kilometres

Figure 4.4 Dissolved uranium content of river, lake and tailings waters of the Elliot Lake district, September 1982. Light numerals represent data for natural waters, bold face for tailings waters. U in ppb.

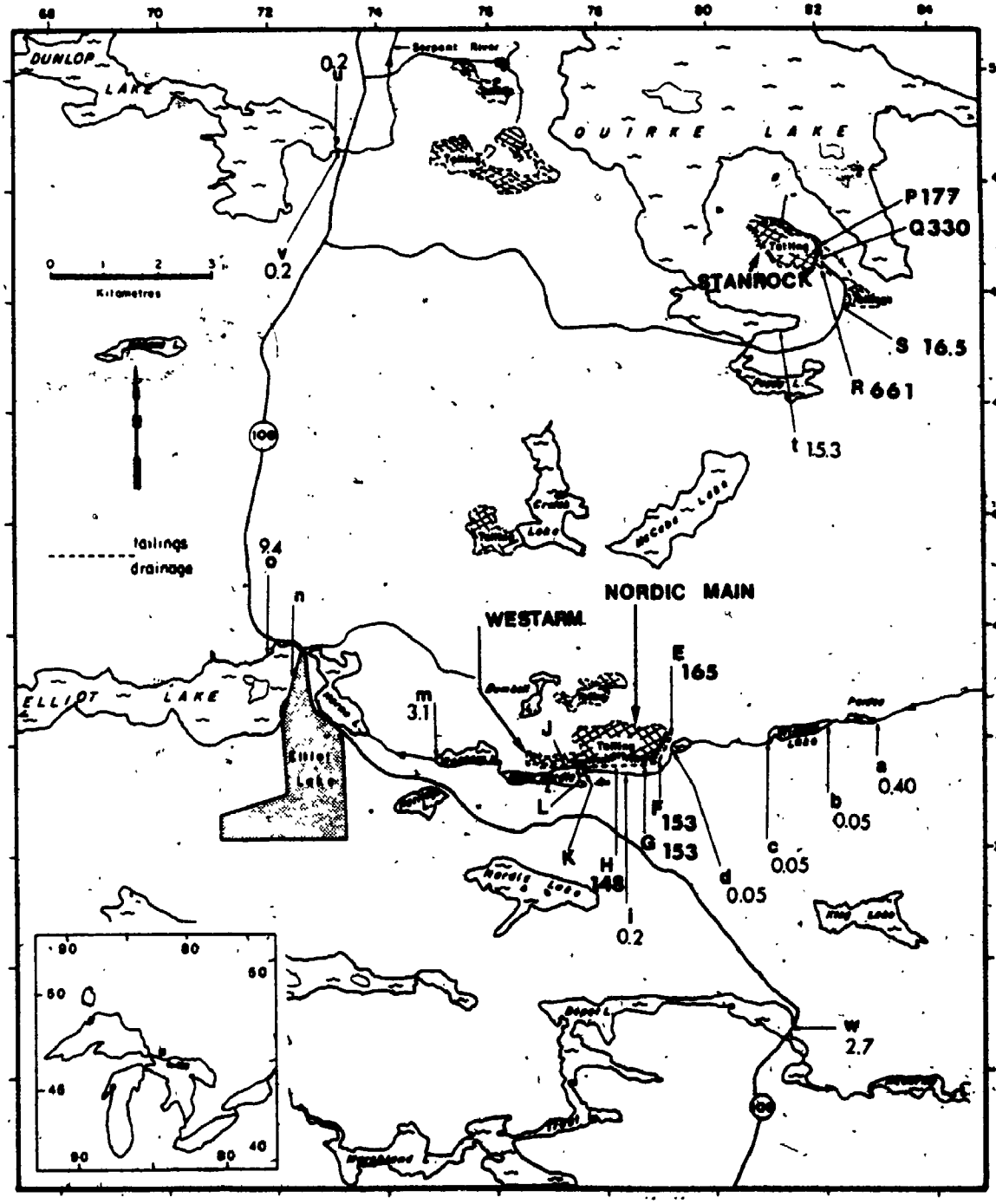

DUNLOP LAKE
Sergeant River
QUIRKE LAKE
STANROCK
P 177
Q 330
S 16.5
R 661
t 15.3
v 0.2
u 0.2
0 1 2 3
Kilometres
tailings
drainage
100
o 9.4
n
WESTARM
NORDIC MAIN
E 165
m 3.1
ELLIOT LAKE
Elliot Lake
J
L
F 153
G 153
K
H 148
I 0.2
a 0.40
b 0.05
c 0.05
d 0.05
W 2.7
17
80
90
60
50
40
30

figure excludes one data point of 7.0 ppb from a stream (station d) located only 200 m east of the Nordic Main tailings impoundment. At this site groundwater contamination from tailings seepage could plausibly account for the unusually high uranium value.

Natural drainage waters of the Serpent river upstream of the Quirke tailings impoundment averages 0.2 ppb (Fig. 4.4, one season only). This figure is closely comparable to the data recorded for Pardee and Stintson lakes. The grand average for dissolved uranium, taken over all natural drainage sampling stations and seasons, is 0.23 ppb ($\pm$ 0.19 1σ). This figure is in accord with the dissolved uranium content of rivers draining the Canadian Shield (Wearich-Verbeek, 1976; Thompson, 1977), but about a factor of three less than the global average riverine aqueous uranium abundance of 0.6 ppb as estimated by Bloch (1980).

Occasional high uranium values were recorded in natural drainage waters remote from tailings. For instance 0.65 ppb U was measured at sampling station c in May 1982 (Fig. 4.3), as against 0.18 and 0.05 ppb for September 1981 and September 1982 respectively (Figs. 4.2 and 4.4). In September 1982 0.4 ppb U was determined for waters at sampling station a, compared to a previous value of 0.1 ppb (Table 4.1). Additional data along with closer sampling intervals will be required to resolve this question.

During the May 1982 sampling period an aliquot of the

Table 4.2. A comparison of U adsorbed onto filtration equipment and U in filtered H_2O, with the two expressed as a percentage. All figures in nanograms.

Sample station	U recovered from apparatus	U determined in H_2O	U recovered/U H_2O - %
A	50	200	25
B	75	400	19
C	225	1,400	16
E	500	118,000	0.4
D	110	980	11
F	550	120,000	0.5
G	200	116,000	0.2
I	235	10,000	2.3
J	150	126,000	0.1
K	1,300	230,000	0.56
O	225	6,000	3.7
T	75	400	19
W	300	3,000	10
X	275	3,800	7
Y	250	1,120	22

two litre volumes collected, and preconcentrated by evaporation prior to determination of uranium by fluorimetry, was also analysed for U by means of neutron activation delayed neutron counting (see Appendix I). In general the results obtained by the two methods were in close agreement (Table 4.1) as was also the case for Thames river waters (see chapter 3).

4.4.2 Problems inherent in the analysis of dissolved uranium at low levels

An important consideration in the analysis of uranium solute concentrations is that of U adsorption from waters on to the filtration apparatus. On site acidification of waters with HNO_3 was conducted in order to avoid this problem in storage bottles. This factor was explored in a series of water samples from Elliot Lake, by washing the filtration glassware in 50% HNO_3 after completion of filtration, evaporating the washings to 10 ml, and analysing for their uranium content (see also Appendix I).

In waters with low dissolved uranium of 0.1 to 0.5 ppb, U recovered from the equipment represented 16 to 25% of the total measured for the waters (Table 4.2). At higher levels of dissolved uranium the absolute quantity of uranium recovered from glassware increased, but represented a smaller proportion relative to that in the water. Visual and microscopic examination of the glassware revealed the

presence of dead algal cells, stranded on and adhered to, the equipment as water levels fell. In view of the known high uranium concentration of algae in the waters being filtered, the uranium recovered in washings is considered to be indigenous to the dead algal cells, rather than to leaching from the glassware or adsorbtion from the filtered waters (see also Appendix I).

Thus the measured dissolved uranium contents of waters are thought to be real, and not significantly perturbed by the filtration process.

4.4.3 Dissolved uranium in tailings waters

Uranium abundances measured in drainage ditch waters carrying the seepage effluent from tailings impoundments is reported in Table 4.1, and mapped out in Figs. 4.2-4.4. Tailings discharge waters arise both from slow dewatering of tailings slurry, as well as from through flow of precipitation landing on the tailings surface (Moffett and Tellier, 1978; Cherry, 1982).

Levels of dissolved uranium in tailings drainage waters exhibit extreme variations, but all are dramatically high when compared to background in natural waters, as discussed above. Values of 56 ppb ($\pm$ 3.5 1σ) [May 1982] to 155 ppb ($\pm$ 7 1σ) [September 1982], were recorded for effluent of the Nordic Main tailings at sampling stations E through F (Table 4.1, Figs. 4.3 and 4.4). These values

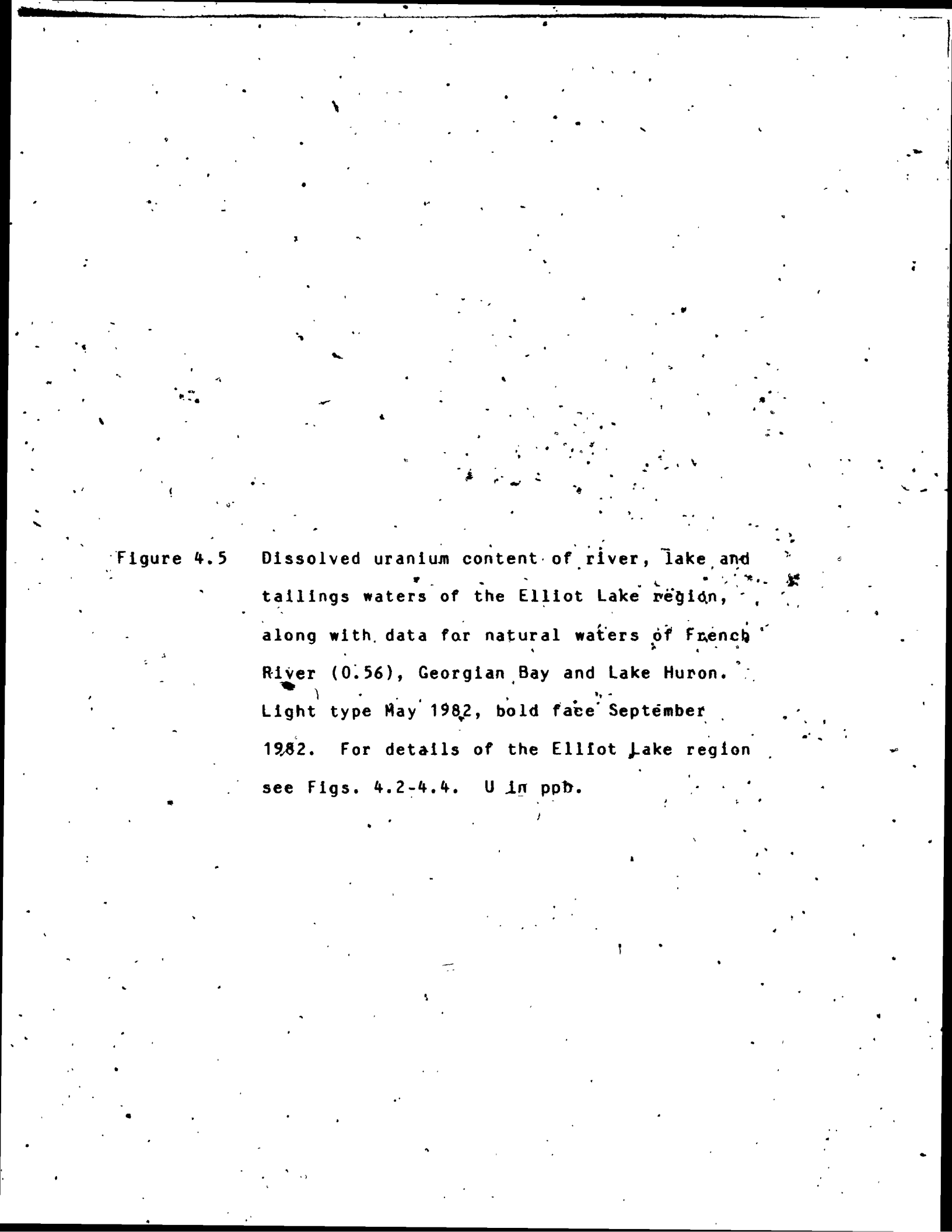
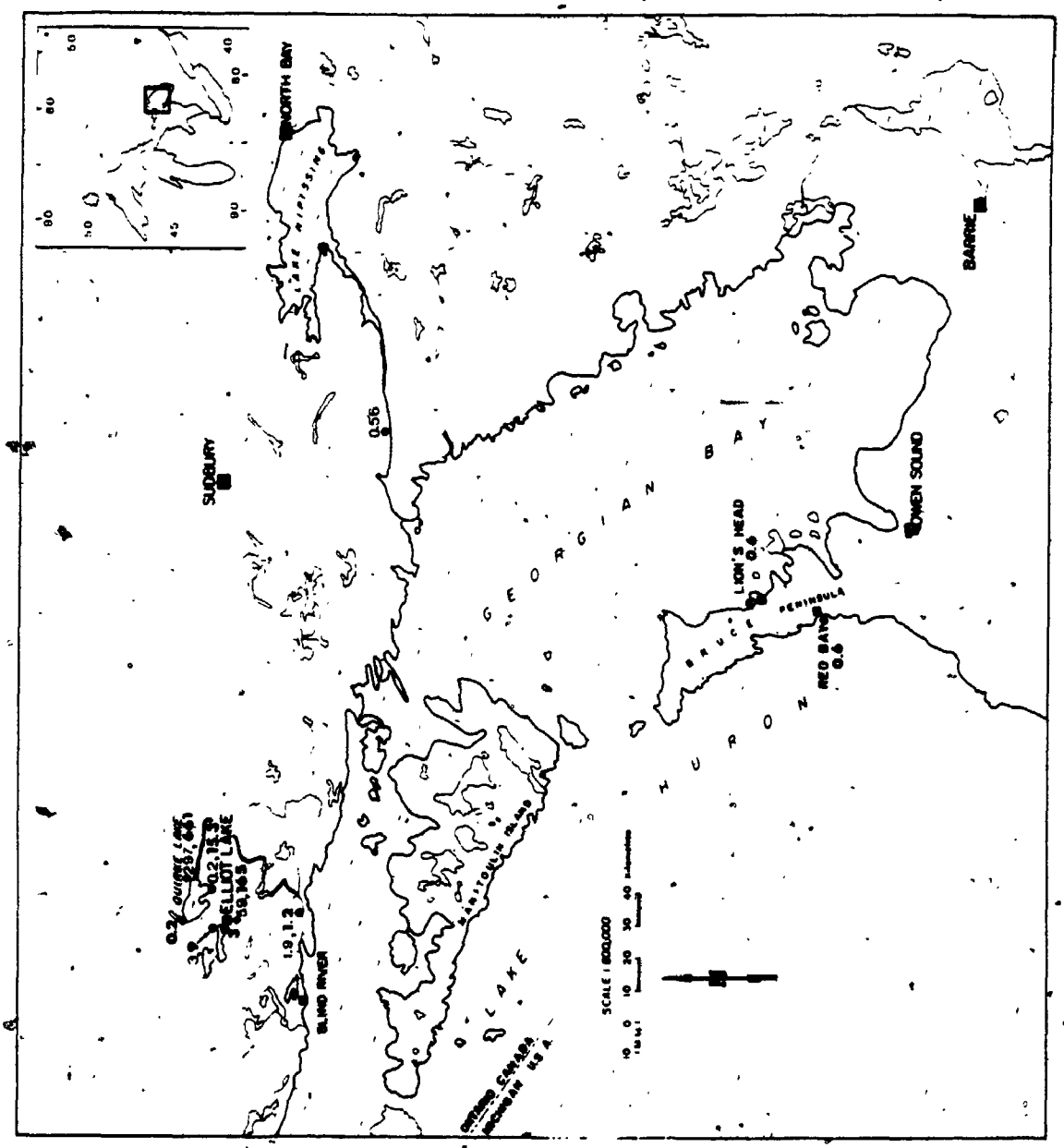


Figure 4.5 Dissolved uranium content of river, lake and tailings waters of the Elliot Lake region, along with data for natural waters of French River (0.56), Georgian Bay and Lake Huron. Light type May 1982, bold face September 1982. For details of the Elliot Lake region see Figs. 4.2-4.4. U in ppb.


NORTH BAY
SUDBURY
BARRIE
OWEN SOUND
LION'S HEAD
PENINSULA
BRUCE
RED BAY
0.6
ELLIOT LAKE
BLIND RIVER
MANITOULIN ISLAND
GEORGIAN BAY
HURON
LAKE
SCALE 1:800,000

represent 240 and 670 times the background aqueous uranium of 0.23 ppb.

For the Westarm tailings seepage waters, uranium levels of 63 (station J) to 580 ppb (station K) were recorded. Corresponding figures for the Stanrock tailings of the Denison mine are 80 (station P) to 660 ppb (station R). The grand averages of dissolved uranium in tailings discharge waters, taken over all sampling stations and seasons are 85 ppb ($\pm$ 62 1σ) [Nordic Main], 230 ppb ($\pm$ 230 1σ) [Westarm] and 273 ppb ($\pm$ 215 1σ) [Stanrock].

The uranium levels in the tailings dam waters located 300 m southeast of the Stanrock impoundment, and receiving the Stanrock discharge, is only 16.5 ppb (Fig. 4.4). This pronounced decrease from 660 ppb in Stanrock discharge is attributable to the partial efficacy of the intervening treatment plant.

Levels of dissolved uranium in tailings effluent as reported above, are fully commensurate with the results of Moffett and Tellier (1978). These authors quoted average uranium solute concentrations of 247, 8,840, and 270 ppb U for low acid class, high acid class, and surface effluent waters respectively.

4.4.4 Dispersion of uranium contamination

The downstream dispersion of anomalous uranium induced by seepage waters from tailings impoundments is mapped out

for the Elliot Lake region in Figs. 4.2-4.4. Uranium levels of up to 115 ppb and 560 ppb in drainage waters of the Nordic Main and Westarm tailings respectively diminish to 29 ppb in North Nordic Lake, to 5 ppb in Westner Lake and 2.4 to 9 ppb in Elliot Lake, the last one being a distance of about six kilometers downstream of the tailings impoundments. In Elliot Lake (n,o), the mean recorded uranium levels of 4.9 ppb ($\pm$ 3.9 1σ) over three seasons (Table 4.1) represents twenty times the background of 0.23 ppb for natural drainage waters above the tailings dams.

Several tens of kilometers downstream of Elliot Lake, waters of the Marshland river carry 1.5 to 2.7 ppb U, still a factor of 6 to 12 times background.

Quirke Lake, which receives treated water escaping from the Stanrock tailings impoundment appears to have fluctuating levels of dissolved uranium. Abundances less than 0.2 ppb were recorded for May 1982, whereas the figure for September 1982 was 16.5 ppb.

The dispersion of anomalous uranium from Elliot Lake over a much wider geographical area is mapped out in Fig. 4.5. Some 50 kilometres downstream of the tailings dams, close to where the Serpent river debouches into Lake Huron, its waters carry 1.2 to 1.9 ppb dissolved uranium. Elsewhere in the Georgian Bay-Lake Huron area natural waters were found to possess a remarkably uniform dissolved uranium content of 0.6 ppb. Thus 50 km below the tailings,

Table 4.3. Abundance of dissolved uranium in Elliot Lake district river, lake and tailings waters, and of uranium in >0.4 μm suspended particulates, at specified sampling stations, 29 August - 2 September 1981. Analyses by fluorimetry.

Sampling station	U-ppb waters	U-ppb >0.4 μm particulates	Particulate weight - mg	Concentration factor particulates/water
Natural drainage above Nordic tailings				
B(1)	0.18	28,500	1.3	160,000
(2)		1,200,000	5.9	6,700,000
C(1)	0.18	6,100	16.8	34,000
(2)	0.18	2,800	21.8	15,000
		1,230	5,912†	6,800
D(1)	7.0	5,100	7.3	730
(2)	7.0	6,300	5.1	900
Tailings drainage - Rio Algom, Nordic-Westarm				
F(1)	10.0	6,100	10.1	610
(2)	7.6	4,500	13.9	590
G(1)	0.87	25,000	11.2	29,000
(2)	0.87	24,000	21.9	28,000
I(1)	0.5	7,700	4.7	15,000
(2)	0.5	31,000	1.2	62,000
J(1)	128	410,000	4.4	3,200
(2)	207	2,700,000	7.1[1]	13,000
K(1)	776	130,000	12.1[1]	170
(2)	378	560,000	9.6[1]	1,500
N(1)	2.4	12,000	3.2	5,000
(2)	1.0	7,900	4.7	7,900

† - filamentous algae

1 - these samples were mixtures of algae plus a small proportion of tailings sand

Figures in parentheses signify duplicate sampling

the Serpent river is seven fold enriched in uranium over its headwaters, and enriched by a factor of two relative to the larger body of water it flows into (Fig. 4.5).

Presumably, the progressively diminishing levels of aqueous uranium in the Serpent river downstream of Elliot Lake reflects dilution of the artificial contamination spike by addition of waters from tributaries which carry normal background levels of uranium. However, the observed trend to lower values may also result from microbiological activity, and this factor is explored in the ensuing section.

Although, at its mouth the Serpent river carries twice the dissolved uranium content of Lake Huron and Georgian Bay waters, this may largely be due to the fact that the bedrock of its drainage basin is intrinsically uraniferous, as well as due to the tailings water contamination. Furthermore, uranium in the Huronian sediments occurs principally as the soluble mineral uraninite, whereas uranium in granitic rocks of the Grenville Province to the east, drained by the French river etc, is not only at a lower absolute level, but is largely hosted in relatively insoluble minerals such as allanite, monazite and zircon.

Table 4.4. Abundance of dissolved uranium in Elliot Lake district river, lake, and tailings waters, and of uranium in >0.4 μm suspended particulates, at specified sampling stations, 10-14 May, 1982. Analyses by fluorimetry.

Sampling station	U-ppb waters	U-ppb >0.4 μm particulates	Particulate weight - mg	Concentration factor particulates/waters
Natural drainage above Nordic tailings				
A	0.10	120,000	0.3	1,200,000
B	0.20	16,000	2.3	80,000
C	0.65	23,100	17.8	3,500
	0.65	8,600	11,800†	13,500
D	0.49		90.1	
Tailings drainage - Rio Algom, Nordic-Westarm				
E	59		160.9	
F	51		262.6	
G	56	80,000	103.4	1,400
	56	25,000	513.6	450
J	63	11,000	373.1	170
K	115	7,100	9.2	62
L	29			
Tailings drainage - Denison Mine Stanrock				
P	80	1,280	413.0	16
Q	93	898	98.2	11
T	<0.2	<2,000	17.8	
Natural drainage downstream of Elliot Lake				
Y	0.56	<13,000	2.9	

† filamentous algae

Table 4.5. Abundance of uranium in Nordic tailings water at sample site L. Elliot Lake, and of uranium in >0.4 μm particulates, May 14, 1982. Analyses by fluorimetry.

Beaker number	U-ppb water	U-ppb >0.4 μm particulates	acid soluble particulate weight - mg[1]	residual particulates weight - mg[2]	acid soluble/ residual particulates[3]	Concentration factor particulates/water[1]
Beaker 1	63	3,000,000	0.1	<0.1		48,000
		1,125,000	0.2	<0.1		1,800
		10,700	117.2	86.2	1.3	170
		89,700	586.3	486.5	1.2	1,400
		10,900	373.1	952.3	0.39	170
Beaker 2		450,000	0.5	<0.1		7,100
		517,000	104.8	<0.1		8,200
		23,000	117.2	612.2	0.19	360
Beaker 3		29,700	10.3	419.0	0.02	470

1 acid soluble particulate weight = total dry weight of >0.4 μm suspended particulates in 1,000 ml tailings water, minus the weight of residual particules, after HNO_3 and $HClO_4$ digestion.

2 residual particulate weight = weight of residual particules after HNO_3 and $HClO_4$ attack of total suspended particulates.

3 this column = weight of 1, divided by weight of 2.

4.5 The uranium complement of suspended particulates and algal communities

4.5.1 Introduction

Uranium abundances in acid (HNO_3 + HCO_4) soluble suspended particulates, along with particulate weight, are reported in Tables 4.3 through 4.11. Suspended particulates of >0.45 μm were recovered during filtration of two litre aliquots of river and tailings waters as described in Appendix I.

This 'screen' size retained mineral particulates, unicellular algae, filamentous algae, diatoms, protozoa, some bacteria and yeast cells, but probably not virus. The relative proportions of inorganic mineral particulates to microorganisms in the suspended fraction is considered further below.

At some locations, communities of prolific filamentous algae, and of unicellular algae either suspended or growing in mats were observed. Such communities of microorganisms were collected en masse, since by virtue of their appreciable weight precise replicate analyses of their uranium and complement of other heavy metals was possible. Data for these prolific algal communities are given in Tables 4.5-4.9, and 4.11, 4.12. A summary of the principal microorganisms present is compiled in Table 4.13.

Table 4.6. Abundance of uranium in Nordic tailings water at sample site K, Elliot Lake, and of uranium in >0.4 μm particulates, May 14, 1982. Analyses by fluorimetry.

Beaker number	U-ppb water	U-ppm >0.4 μm particulates	acid soluble particulate weight - mg[1]	residual particulate weight - mg[2]	acid soluble/ residual particulates[3]	Concentration factor particulates/water[1]
Beaker 1	115	43,200	16.2	<0.1		380
		237,000	2.0	<0.1		2,100
		110,500	4.3	<0.1		960
		77,300	9.7	<0.1		670
		19,700	48.1	56.7	0.85	170
		83,500	18.1	30.7	0.59	730
Beaker 2		165,000	0.7	<0.1		1,400
		59,800	16.3	16.9	0.96	520
		41,400	741.9	541	1.4	360
		118,000	1,398	5,976	0.23	1,030
Beaker 3		348,000	297.0	1,325	0.22	3,000

1 acid soluble particulate weight = total dry weight of >0.4 μm suspended particulates in 2,000 ml water, minus the weight of residual particules, after HNO_3 and $HClO_4$ digestion.

2 residual particulate weight = weight of residual particules after HNO_3 and $HClO_4$ attack of total suspended particulates.

3 this column = weight of 1, divided by weight of 2.

4.5.2 Uranium in suspended particulates and algal communities: natural waters

The essential feature of the results for suspended particulates is that their uranium content is 10^4 to 10^5 times that of the host river waters. Measured uranium abundances in algae, calculated relative to dry weight, ranged from a low of 1,000 ppb to a high of 1,200,000 ppb (Table 4.3). Despite these extreme values, the bulk of the data fell in a relatively narrow range, averaging 19,300 ppb ($\pm$ 28,300 1σ) [excluding the extreme upper value, Tables 4.3, 4.4, 4.10].

Concentration factors, defined as uranium abundance in particulates divided by that in the water, averaged 177,000 ($\pm$ 280,000 1σ) for September 1981, 67,800 ($\pm$ 59,200 1σ) for May 1982, and 165,000 ($\pm$ 215,000 1σ) for September 1982, for Pardee and Stintson Lakes. For the Serpent river upstream of the Quirke tailings, the concentration factor averaged 75,700 ($\pm$ 61,300 1σ) in September 1982. Although there exists a significant spread between individual data points, the average concentration factors are rather uniform, all lying within a factor of four of the lowest.

The average mass of acid soluble water-borne suspended particulates, averaged over all sampling stations and seasons, was 11.2 $\pm$ 21.3 mg 1σ per litre (17 data points). Based on a mean uranium abundance of 19,300 ppb, it is clear that this particulate fraction carries a significant

proportion [11.2 mg/l x 19,300 ppb = 216 ng U/l] of the total riverine uranium flux, representing about 45% of the total or 99 percent of that transported directly in solution [10^3 g x 0.23 ppb = 230 ng U/l].

Given the basic uniformity of average results for the different locations and sampling seasons, the data are collectively interpreted in terms of a relatively constant partitioning behaviour between dissolved uranium and that indigenous to water borne suspended particulates, composed chiefly of microoorganisms. The partion coefficient, Kd = U (microorganisms)/U(H_2O) for this part of the natural drainage system is about 8.4×10^4. This result is in accord with partition coefficients calculated for experiments reported in chapter 2, and with the K_d of 2×10^4 estimated for suspended microorganisms of the upper Thames river. Whereas concentration factors refer to individual water samples, Kd is taken to represent the bulk suspended biomass to water uranium partitioning, integrated over the specified drainage system.

Large communities of filamentous algae, each in the 10 kg range, were observed at site c, below the western extremity of Stintson Lake, on all three sampling seasons (Plate 4.1). Similar growths of filamentous algae were recorded from site v, on the Serpent river immediately below Dunlop Lake. These particular filamentous algal communities apparently favored stagnant waters.

Plate 4.1 Upper. Prolific growths of filamentous algae in stagnant waters, location c, Stintson Lake (see Fig. 4.1). Horizontal field of view 4 metres.

Lower. Photomicrograph of filamentous algae Ulothrix sp. illustrated above. Magnification 400 diameters.

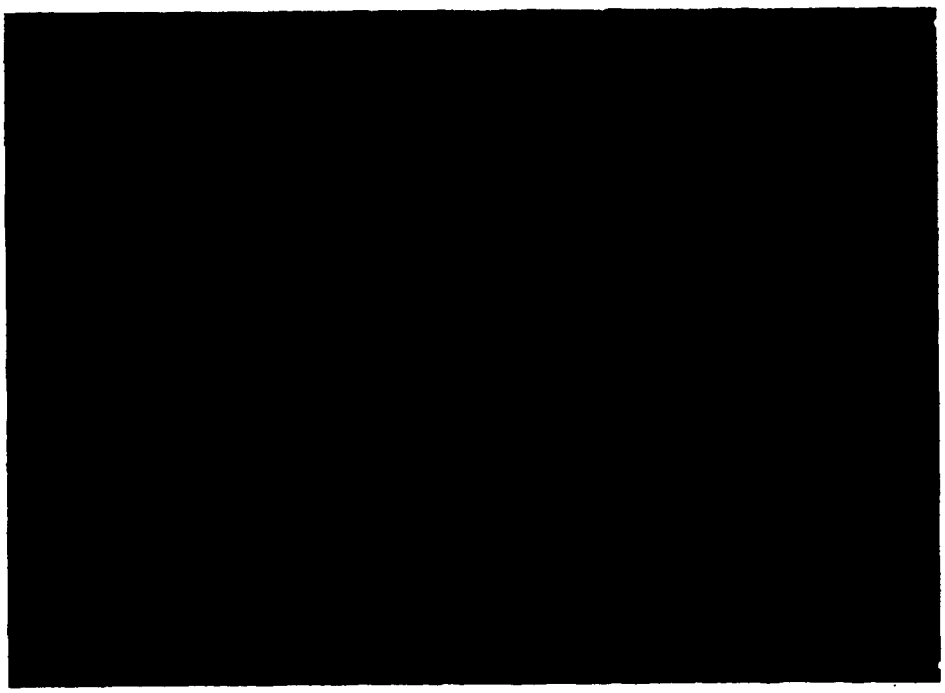


Table 4.7. Abundance of uranium in Nordic tailings water at sample site L, Elliot Lake, and of uranium in >0.4 μm particulates, May 14, 1982. Analyses by fluorimetry.

Beaker number	U-ppb water	U-ppb >0.4 μm particulates	acid soluble particulate weight - mg[1]	residual particulate weight - mg[2]	acid soluble/ residual particulates[3]	Concentration factor particules[1]/water
Beaker 1	29	189,000	0.7	<0.1		6,600
		974,000	0.7	<0.1		34,000
		750,000	0.4	<0.1		26,000
		681,000	1.3	<0.1		23,000
		350,000	0.1	<0.1		12,000
		509,000	1.5	<0.1		18,000
		88,200	29.2	2.2	130	3,400
		264,000	41.2	12.4	3.3	9,100
		223,000	280.5	48.9	5.7	7,700
Beaker 2		250,000	0.8	<0.1		8,600
		138,000	6.7	<0.1		4,800
		141,000	68.9	7.9	8.7	4,800
Beaker 3		200,000	20.0	<0.1		6,900
Beaker 4		66,100	9.8	<0.1		2,300
		144,000	72.8	<0.1		5,000
Beaker 5		211,000	6.5	<0.1		7,300
		39,200	5.1	<0.1		1,300
		150,000	4.5	<0.1		5,200
		138,000	464.4	<0.1		4,700

1 acid soluble particulate weight = total dry weight of >0.4 μm particulates minus the weight of residual particules, after HNO_3 and $HClO_4$ digestion.

2 residual particulate weight = weight of residual particules after HNO_3 and $HClO_4$ attack of total particulates.

3 this column = weight of 1, divided by weight of 2

Table 4.8. Abundance of uranium in Stanrock tailings water at sample site, P, Elliot Lake, and of uranium in >0.4 μm particulates, May 14, 1982. Analyses by fluorimeter.

Beaker number	U-ppb water	U-ppb >0.4 μm particulates	acid soluble particulates weight - mg[1]	residual particulate weight - mg[2]	acid soluble/ residual particulates[3]	Concentration factor particulates/water[1]
Beaker 1	80	22,400	13.4	<0.1		280
		147,000	2.2	<0.1		1,800
		85,900	3.2	<0.1		1,100
		50,900	5.4	<0.1		640
		88,200	3.4	<0.1		1,100
		11,000	3.4	<0.1		140
		90,800	2.5	<0.1		1,100
		51,700	5.8	<0.1		650
		62,500	4.8	<0.1		780
		119,000	3.3	<0.1		1,500
		175,000	2.0	<0.1		2,500
		152,000	2.3	<0.1		1,900
		114,000	3.3	<0.1		1,400
		130,000	2.7	<0.1		1,600
		214,000	3.7	<0.1		2,700
		76,100	4.6	<0.1		950
		160,000	3.9	<0.1		2,000
		33,800	14.1	<0.1		420
		8,030	2,988.3	1,008	2.96	100
Beaker 2		141,300	2.3	<0.1		1,800
		272,000	1.1	0.8		3,400
		8,210	244.2	<0.1		100
		21,900	26.2	<0.1		270
		14,800	18.6	<0.1		180
		7,600	2,786.6	88.2	31	95
		3,130	1,169.9	2,582	0.45	40
		1,083	793.4	1,486	0.53	13
Beaker 3		55,500	24.3	37.6	0.65	690

1 acid soluble particulate weight = total dry weight of >0.4 μm particulates minus the weight of residual particules, after HNO_3 and $HClO_4$ digestions.

2 residual particulate weight = weight of residual particules after HNO_3 and $HClO_4$ attack of total particulates.

3 this column = weight of 1, divided by weight of 2.

Uranium concentrations in the filamentous algae ranged from 1,230 to 8,600 ppb (site c), and from 1,090 to 8,020 ppb for site v, with a grand average of 4,500 ± 3,520 ppb 1. (Tables 4.3, 4.4, 4.10). This is a factor of four less than the average for their suspended unicellular counterparts, a function either of species specificity for uranium uptake and/or the fact that the surface area/volume ratio of filamentous algae is clearly less by several orders of magnitude than that of unicellular species.

4.5.3 Problems encountered in determining particulate U

From inspection of data for the uranium abundance of suspended particulates (Tables 4.3-4.11) a broad inverse relationship is apparent between calculated uranium levels and the measured mass of soluble particulates. This feature was also evident for suspended particulates in Thames river waters where two explanations were offered for the effect (see section 3.5.1; Figs. 3.15-3.17). First, a bias may operate whereby small particulate masses of 0.1 mg are underestimated via weight (loss or gain) of the filter paper itself during drying or handling, given that the filter paper is ∿10,000 times such measured particulate masses. Second, for those samples where a significant inorganic mineral component was present (and for which the latter was also assumed to possess an approximately average crustal U of 3 ppm) the HNO_3 + $HClO_4$ digestion procedure

leached a significant mass, but relatively minor proportion of the total uranium, from the partially soluble inorganic minerals. This would act to increase the apparent mass of the acid soluble (microorganism) component whilst at the same time depressing its estimated uranium abundance. Clay quartz, and feldspar minerals positively identified by X-ray diffraction in the >0.45 μm particulates from Elliot Lake waters are certainly soluble to some extent in boiling perchloric plus nitric acids.

Whilst it is difficult to assess the possible influence of the first mentioned factor, the data in Tables 4.4-4.11 definitely help to qualitatively estimate the second one. For example, in Tables 4.8 and 4.9 the uranium content measured on aliquots of algae identified microscopically as Euglena, and successfully purified by washing from Euglena-sand mixtures, is relatively uniform where the weight of residue is effectively zero. However, for the last washings from individual beakers, where silt became entrained with Euglena cells, the residual particulate weight becomes significant. For these samples, the calculated uranium content of Euglena fell by a factor of ten relative to essentially pure cultures (Tables 4.8, 4.9).

These results provide evidence that inorganic minerals admixed with microorganisms act to depress the measured uranium abundance of the microorganisms in the manner suggested above. A corollary to this is that the inorganic

fraction has a relatively minor uranium content relative to the microorganisms, even in tailings waters at Elliot Lake. Thus the calculated uranium complement of suspended nonorganic particulates are probably minimal, at least for those with appreciable masses of ≥ 1 mg/litre.

Ideally, suspended particulates should be recovered by on site centrifuging of waters that were not acidified, and separating the microorganisms from minerals by further means.

4.5.4 Uranium in suspended particulates: tailings waters

The uranium content of suspended particulates in tailings discharge is 10^3 to 10^5 times that of the host waters. This result is in accord with the magnitude of uranium concentration for suspended particulates in natural drainage, despite the fact that tailings waters are highly contaminated with many apparently toxic metal species (Table 4.12).

Measured uranium abundances in the water borne suspended particulate fraction, which was composed chiefly of algae (Table 4.13), ranged from a low of 900 ppb (Table 4.4) to a high of 2,700,000 ppb (Table 4.12). The latter figure is equivalent to 0.27% uranium by dry weight.

Extreme differences in the uranium complement of particulates were noted both between discharge waters from different tailings impoundments, and from a given drainage

Table 4.9. Abundance of uranium in Stanrock tailings water of sample site Q, Elliot Lake, and of uranium in >0.4 μm particulates, May 14, 1982. Analyses by fluorimetry.

Beaker number	U-ppb water	U-ppb >0.4 μm particulates	acid soluble particulate weight - mg[1]	residual particulate weight - mg[2]	acid soluble/ residual particulates[3]	Concentration factor particulates/water
Beaker 1	93	121,000	6.6	<0.1		1,300
		112,000	8.5	<0.1		1,200
		114,000	4.8	<0.1		1,200
		180,000	2.5	<0.1		1,900
		104,000	7.2	<0.1		1,100
		118,000	7.4	<0.1		1,300
		204,000	2.7	<0.1		2,200
		2,360,000	0.9	<0.1		25,000
		98,400	55.1	17.6	3.1	1,100
		63,900	204	933	0.22	690
Beaker 2		362,000	2.9	<0.1		3,900
		3,040	873	415	2.1	33
Beaker 3		960	6,780	306	21	10
Beaker 4		12,500	330	130	2.5	130
Beaker 5		125,000	1.6	<0.1		1,300
		11,100	1,853	1,436	1.3	120
Beaker 6		51,900	5.3	<0.1		560
		4,425,000	4.0	14.0	0.28	48,000
Beaker 7		166,000	1.5	4.0	0.37	1,800
		15,000	2,298	1,996	1.1	160

1 acid soluble particulate weight = total dry weight of >0.4 μm particulates minus the weight of residual particules, after HNO_3 and $HClO_4$ digestion.

2 residual particulate weight = weight of residual particules after HNO_3 and $HClO_4$ attack of total suspended particulates.

3 this column = weight of 1, divided by weight of 2.

Table 4.10. Abundance of uranium in river, lake and tailings water, and of uranium in >0.4 μm suspended particulates at specified sampling stations, vicinity of Elliot Lake, September 8-10, 1982. Analyses by fluorimetry.

Sampling station	U-ppb water	U-ppb >0.4 μm particulates	acid soluble particulate weight - mg[1]	residual particulates weight - mg[2]	acid soluble/ residual particulates[3]	Concentration factor particulates/water
Natural drainage above Nordic tailings						
A	0.4	3,230	6.2	<0.1		8,100
B	0.05	3,850	5.2	<0.1		77,000
C	0.05	20,400	2.7	<0.1		410,000
D	0.05	<5,200	2.3	<0.1		
I	0.2	20,800	1.8	<0.1		100,000
Tailings drainage - Rio Algom						
E	165	130,000	40.4	16.3	2.5	790
F	153	144,000	49.4	14.1	3.5	940
H	148	67,500	38.9	8.7	4.5	450
M	3.1	190,000	0.5	<0.1		61,000
Natural drainage above Quirke tailings						
U	0.2	19,000	2.1	0.3	7.0	95,000
V	0.2	25,000	0.8	<0.1		125,000
W	2.7	19,000	2.1	0.7	3.0	7,000
Tailings drainage - Denison Mine						
P	177	183,000	0.3	<0.1		1,000
Q	330	136,000	0.7	<0.1		410
R	661	517,000	5.8	1.6	3.6	780
S	16.5	57,100	0.7	<0.1		3,500
T	15.3	14,300	1.4	<0.1		930
Natural drainage downstream of Elliot Lake						
X	1.2	13,300	3.0	<0.1		11,000
Z	0.6	18,600	2.6	<0.1		28,000
ZZ	0.6	50,000	0.4	<0.1		83,000

1 acid soluble particulate weight = total dry weight of >0.4 μm suspended particulates in 2,000 ml water minus the weight of residual particules, after HNO_3 and $HClO_4$ digestion.

2 residual particulate weight = weight of residual particules after HNO_3 and $HClO_4$ attack of total suspended particulates.

3 this column = weight of 1, divided by weight of 2.

Figure 4.6 Plot of uranium concentrations in various algae versus the dissolved uranium content of their aqueous medium. All open symbols are data for natural waters, closed symbols refer to tailings waters as illustrated in Fig. 4.1. Tie lines between solid diamonds relate to averages based on Euglena samples with no admixed inorganic mineral matter (right hand diamond), and for all Euglena data (left hand diamond) [see Table 4.12].

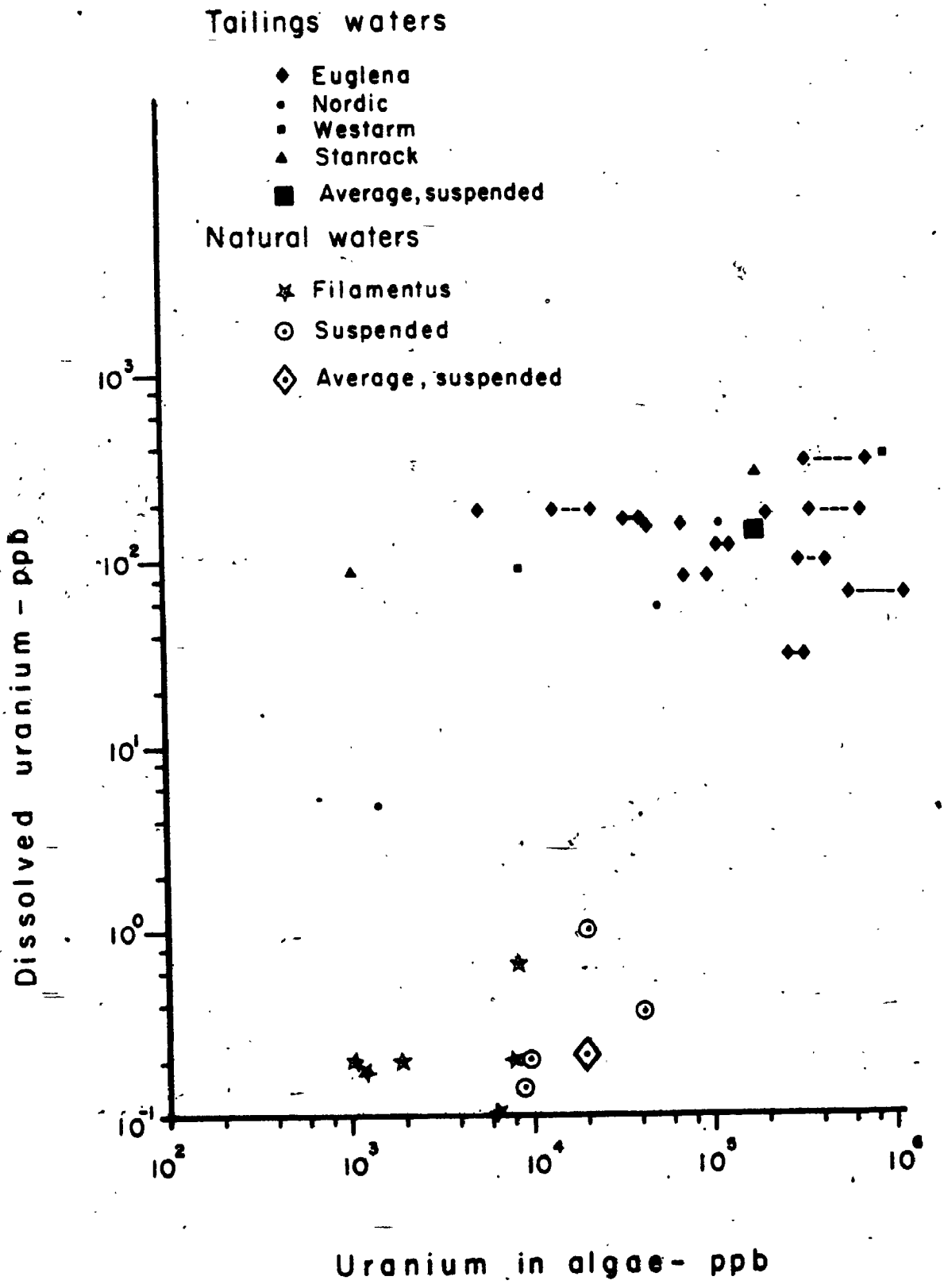
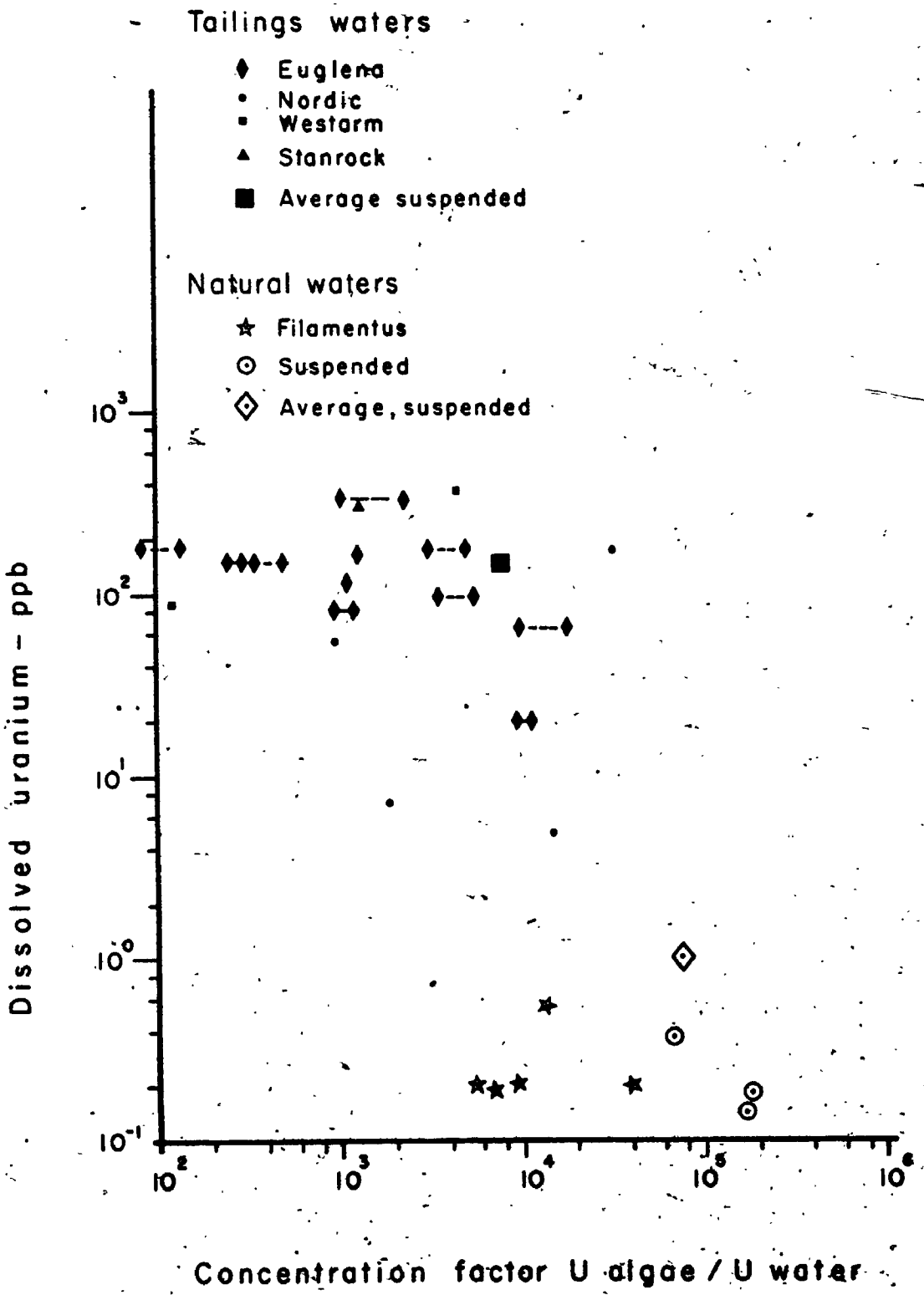

Tailings waters
Euglena
Nordic
Westarm
Stanrock
Average, suspended
Natural waters
Filamentus
Suspended
Average, suspended
Dissolved uranium - ppb
Uranium in algae - ppb
10^3
10^2
10^1
10^0
10^-1
10^2
10^3
10^4
10^5
10^6

Figure 4.7 Plot of concentration factors for various algae versus the dissolved uranium content of their aqueous medium. All open symbols are data for natural waters, closed symbols refer to tailings waters. Tie lines between solid diamonds relate to averages based on Euglena samples with no admixed inorganic mineral matter (right hand diamond), and for all Euglena data (left hand diamond) [see Table 4.12]. Concentration factor defined as the uranium abundance of algae divided by the dissolved uranium content of waters.


Tailings waters
Euglena
Nordic
Westarm
Stanrock
Average suspended
Natural waters
Filamentus
Suspended
Average, suspended
Dissolved uranium - ppb
10^3
10^2
10^1
10^0
10^{-1}
10^2
10^3
10^4
10^5
10^6
Concentration factor U algae / U water

ditch over separate sampling seasons. For instance, suspended particulates from the Nordic, Westarm, and Stanrock tailings seepage averaged 52,500 ppb (± 38,900 1σ), 9,050 ppb (± 2,760 1σ) and 1,100 ppb U (± 270 1σ) during the May 1982 sampling time. In the Nordic tailings discharge, uranium contents of the particulate fraction averaged 14,900 ppb (± 13,600 1σ), 52,500 ppb (± 38,900 1σ), and 110,000 ppb (± 66,500 1σ) for the three sampling seasons respectively. At the Denison Mine, particulates in discharge from the Stanrock impoundment carried 1,100 ppb U (± 270 1σ) and 181,000 ppb (± 198,000 1σ) for May and September 1982 respectively (Fig. 4.6).

The grand average uranium complement of suspended particulates in tailings waters, taken over all sampling stations and seasons, is 184,000 ppb (± 358,000 1σ). This figure represents about ten times the uranium abundance of 19,300 ppb in natural counterparts (see section 4.5.2, and Fig. 4.6). For tailings waters, the grand average of 147 ppb (± 180 1σ) is 640 times the mean background level of 0.23 ppb in natural waters. Tailings waters carry a suspended particulate load averaging 83.1 mg/l, a figure significantly higher than the mean of 11.2 mg/l for natural drainage. This is thought to be at least partly due to an overall higher water velocity in the drainage ditches as against natural lakes and streams described above.

Concentration factors, defined as the uranium abund-

Table 4.11. Abundance of uranium in river, lake and tailings waters, and of uranium in algae, at specified sampling stations, vicinity of Elliot Lake. September 8-10, 1982. Analyses by fluorimetry.

Sampling station	U-ppb water	U-ppb algae	acid soluble particulate[1] weight - mg	residual particulate[2] weight - mg	acid soluble/ residual particulates[3]	Concentration factor algae/water
Natural drainage above Nordic tailings						
C†	0.05	6,450	2,683	243	11.0	130,000
Tailings drainage - Rio Algom						
E	165	206,000	799	187	4.2	1,200
F 1	153	5,260	426	<0.1		34
2		20,500	173	<0.1		134
3		84,200	925	<0.1		550
4		59,200	1,992	10.7	186	387
H 1	148	2,000	502	6.7	75	14
2		11,300	839	<0.1		76
3		81,100	280	<0.1		548
4		69,200	1,575	117	13.5	467
5		118,000	796	<0.1		797
6		35,900	1,183	249	4.7	242
Natural drainage above Quirke tailings						
V 1†	0.2	1,910	5,499	372	14.8	9,500
2†		8,020	3,867	159	24.3	40,000
3†		1,090	923	46	20.1	5,400
Tailings drainage - Denison Mine						
PI 1	177	69,900	160	<0.1		395
2		9,270	47.7	<0.1		52
3		14,300	951	16.3	58.3	81
4		2,660,000	23.5	<0.1		15,000
5		7,082	83.3	<0.1		40
6		6,800	694	113.0	6.1	38
7		12,900	506	29.1	17.3	73
		81,400	282	57.8	4.9	460
Tailings drainage - Denison Mine						
PII 1	177	5,380	823	<0.1		30
2		5,090	191	<0.1		29
3		8,150	203	<0.1		46
4		4,300	117	<0.1		24
5		4,410	614	<0.1		25
6		5,970	282	<0.1		34
7		3,610	482	9.8	49	20
8		11,080	612	26.4	23	62
9		5,190	648	119.8	5.7	29

Table 4.11. Continued.

Sampling station	U-ppb water	U-ppb algae	acid soluble particulate weight - mg[1]	residual particulate weight - mg[2]	acid soluble/ residual particulates[3]	Concentration factor algae/water
Tailings drainage - Denison Mine						
PIII 1	177	28,100	715	<0.1		159
2		7,040	1,037	374	2.8	40
3		5,330	3,769	598	6.3	30
4		15,600	280	<0.1		88
5		28,100	2,510	183	14	159
6		17,900	312	17.7	18	101
7		26,400	355	<0.1		149
8		1,840	16.3	0.9	18	108
9		2,810	37.4	1.3	29	16
10		7,890	6,576	817	8.0	45
QI 1	330	20,000	715	<0.1		61
2		57,700	1,695	17.8	95	175
3		59,000	600	9.9	61	179
4		1,280,000	120	<0.1	15	3,880
QII 1	330	75,000	3,434	163	21	229
2		45,000	1,676	93	18	136
3		28,000	158	<0.1		85
QIII 1	330	12,800	113	<0.1		39
2		53,000	1,425	17.6	81	161
3		692,000	1,070	21.8	49	2,100
QIV 1		3,080,000	3.6	<0.1		9,300
2		86,000	246	<0.1		261
3		34,500	10,250	114.6	89	104

1. acid soluble particulate weight - total dry weight of >0.4 μm suspended particulates in 1,000 ml tailings water, minus the weight of residual particulates, after HNO_3 and $HClO_4$ digestion.
2. residual particulate weight = weight of residual particules after HNO_3 and $HClO_4$ attack of total suspended particulates.
3. this column - weight of 1, divided by weight of 2.

† filamentous algae.

ance in particulates divided by that in water, are plotted in Fig. 4.7. Wide variations in concentration factors exist, from 120 to 14,600 and an overall inverse relationship is apparent between the level of dissolved uranium and concentration factor (Fig. 4.7). It is clear that the grand average of concentration factors for suspended particulates in tailings waters at 7,700 (± 12,100 1σ) is much lower than that for equivalents in natural waters (Fig. 4.7).

Given a mean uranium abundance of 184,000 ppb for the acid soluble suspended particulates, it is clear that this fraction carries a significant proportion [83.1 mg/l x 184,000 ppb = 15,300 ng U/l] of the total tailings uranium flux, representing about 9% of the total, or 10% of that transported directly in solution [10^3 g x 147 ppb = 147,000 ng U/l].

The extreme scatter in results for both uranium abundance and concentration factor in the particulates of tailings waters is considered to be the result of at least two factors. First, real variations in the dissolved uranium content of tailings waters at different sites along the drainage ditches, and at different seasons (see Table 4.1, Figs. 4.2-4.4). Second, the influence of inorganic suspended particulates and/or errors of weight measurement, on the calculated uranium abundance of the acid soluble organic fraction.

In relation to the first factor, results for the Elliot lake region (Tables 4.1-4.11) as well as for the Thames river and experiments reported in chapter 2, reveal that the absolute uranium abundance in acid soluble suspended particulates (largely algae) generally correlates with higher levels of dissolved uranium in the aqueous medium. This effect is illustrated in Fig. 4.6. For instance at the Stanrock tailings, the uranium content of suspended particulates diminished from 517,000 ppb through 183,000 ppb to 14,300 ppb as the aqueous uranium dropped from 661 through 177 to 153 ppb. The last figure is from a station (t) ∿2 km downstream of the tailings.

Although there is not a strict correspondence of particulate and aqueous uranium abundances, partly due to other sources of uncertainty, an overall trend is clear. In this instance, the scatter in results for uranium concentration of particulates reflect a real variation. The second factor introducing variability to the concentration data, namely that of inorganic suspended particulates, was discussed in section 4.5.3.

4.5.5 Uranium in algal communities: tailings waters

An astounding feature of tailings waters at Elliot Lake is the presence of extensive Euglena algae. These algal growths are prolific even at the immediate base of tailings dams, where the discharged seepage is most pollut-

Plate 4.2 Upper. Discharge waters from the Nordic Main tailings impoundment, station H (Fig. 4.1). These waters contain prolific Euglena elastica.

Lower. North Nordic lake viewed from station L. Prolific growths of Phaeaster sp. on the lake bottom, show through the reddish-brown coloration induced by suspended iron oxides. Waste dump and Rio Algam mill in the background.

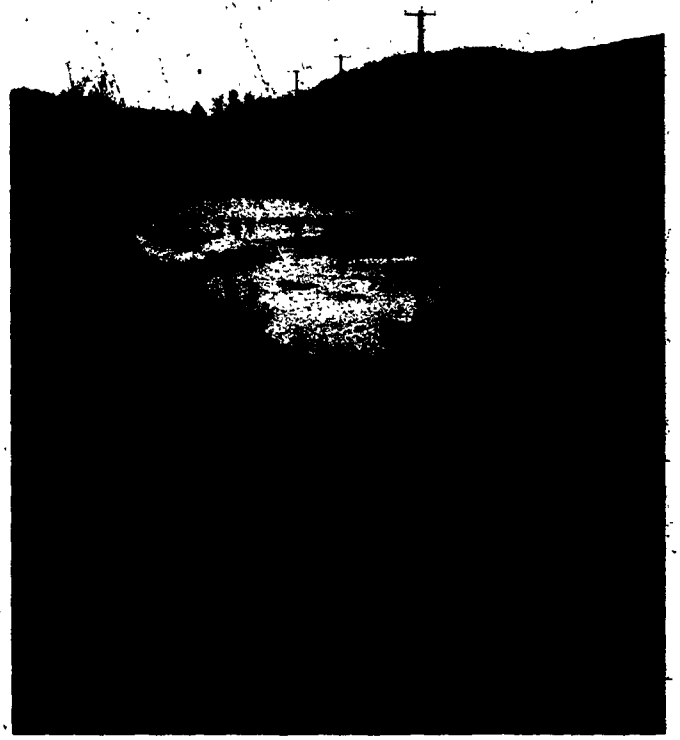


Plate 4.3 Prolific growth of Euglena in 'carpets' on the bottom of a tailing drainage ditch, 250 m from the Stanrock impoundment, station P. Horizontal field of view about 40 m.

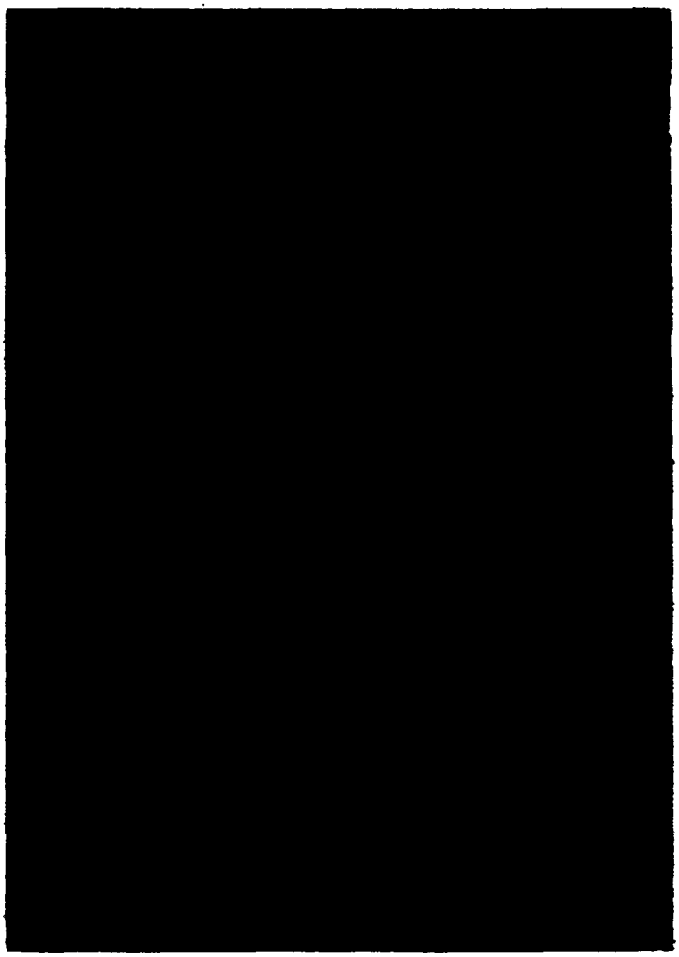
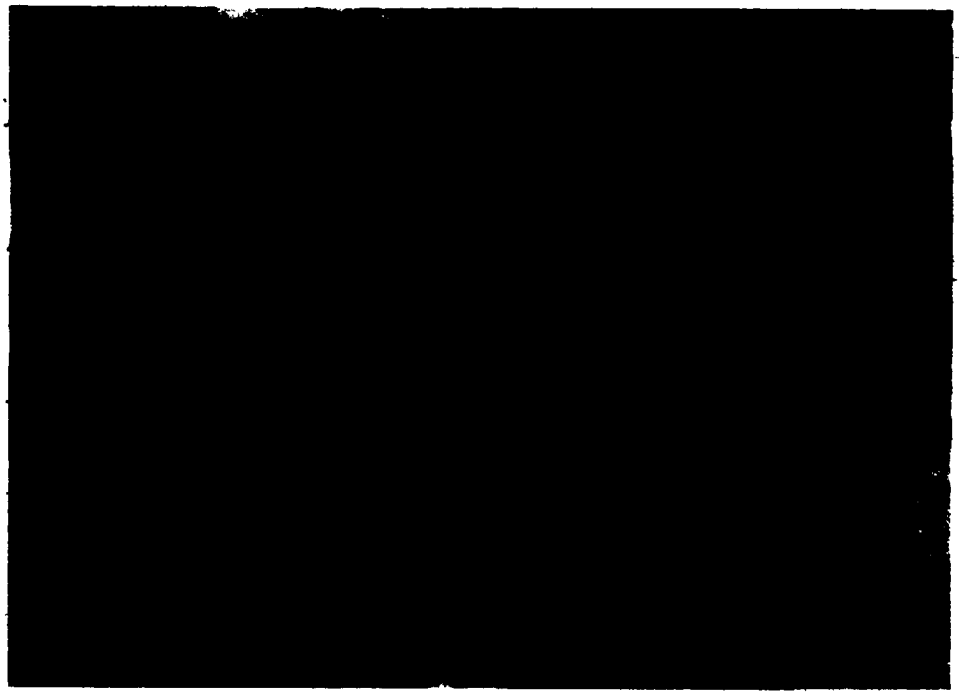
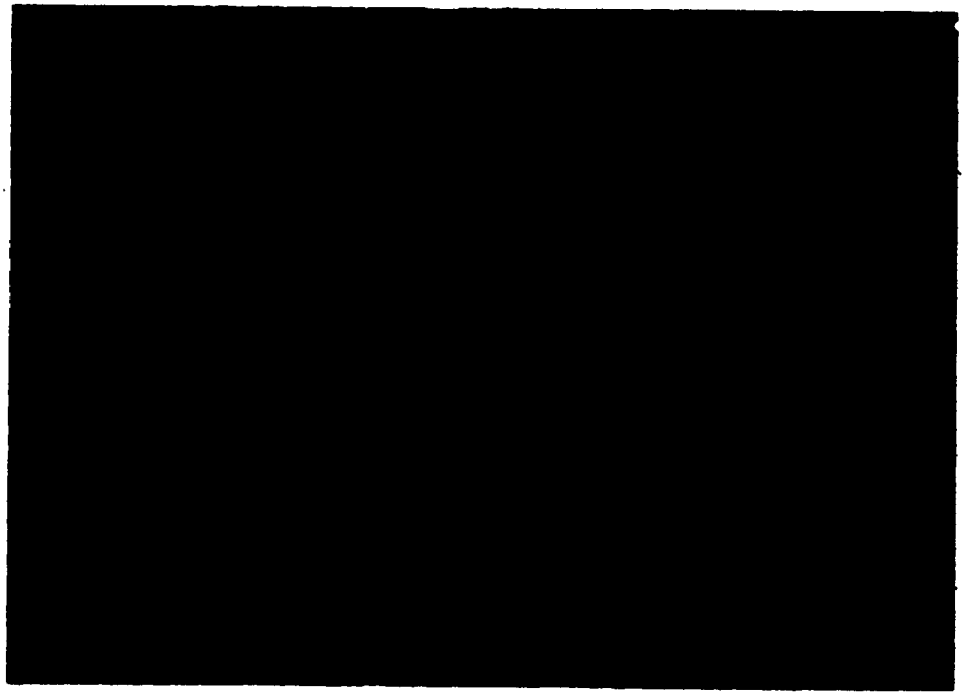


Plate 4.4 Upper. Prolific growths of Euglena elastica on the bottom of a tailings drainage ditch 250 m from the Stanrock impoundment, station P. Horizontal field of view 0.6 metres.

Lower. Photomicrograph of Euglena elastica illustrated above. Magnification 200 diameters.





ed and pH lowest (Plates 4.2-4.4). Unlike the filamentous algae described above, which are restricted to natural stagnant waters, Euglena was observed in relatively still waters as well as in fast flowing discharge. Communities of microorganisms dominated by Euglena developed either as mats carpeting the base of drainage ditches (cf. stations F, G, J, L, P, Q, Figs. 4.1, Plate 4.2), or as suspensions in quieter waters such as at station H (Fig. 4.1). Euglena communities were sampled and separated from sandy bottom material they were attached to for analysis of their uranium content, as described in Appendix I.

Multiple analyses of uranium concentration in Euglena colonies are reported in Tables 4.5-4.11 and averages compiled in Table 4.12. Average concentrations are given for groups of subsamples (a) where no detectable inorganic material coexisted with the Euglena and, (b) for all data inclusive of those with admixed minerals. In general, the two averages are comparable, the latter typically being lower than the former, but by less than a factor of two (see section 4.5.3; Figs. 4.6, 4.7).

Average uranium concentrations in individual communities of Euglena ranged from 42,300 ($\pm$ 36,000 1σ) to 1,127,000 ppb ($\pm$ 1,191,000 1σ), the second figure corresponding to 0.127% U by dry weight. Given such high levels of uranium, these Euglena present an interesting possibility for future TEM, SEM and EDS analyses of uranium siting

4.12. Summary of data for average uranium contents of Euglena in tailings waters of the Elliot Lake region. For sample locations see Fig. 4.1.

Sampling time	Sampling location	U-ppb H_2O	U-ppb algae ± 10	number of observations	Concentration factor
May 1982	L	63	1,127,000 (±1,190,000)†	4	17,800
			584,000 (±979,000)*	9	9,270
May 1982	K	115	127,000 (±76,300)	5	1,100
			118,000 (±98,300)	11	1,030
May 1982	L	29	319,000 (±280,000)	15	11,000
			290,000 (±256,000)	19	9,900
May 1982	P	80	97,500 (±69,700)	23	1,220
			75,900 (±67,300)	28	950
August 1982	Q	93	317,000 (±619,000)	13	3,400
			428,000 (±1,070,000)	20	4,600
September 1982	E	165	206,000	1	1,250
	F	153	36,000 (±41,900)	3	240
			42,300 (±36,000)	4	280
	H	148	70,100 (±54,200)	3	474
			35,200 (±33,100)	6	238
	PI	177	687,000 (±1,320,000)	4	3,880
			357,000 (±931,000)	8	2,020
	PII	177	5,500 (±1,420)	6	31
			5,910 (±2,300)	9	33
	PIII	177	23,400 (±6,780)	3	132
			14,100 (±10,600)	10	80
	Q	330	751,000 (±1,240,000)	6	2,280
			336,000 (±84,300)	13	1,020

† - of each sampling station the upper figure represents the average of measurements for Euglena with no admixed inorganic minerals.

* - the latter figure is for all data, including measurements for Euglena with an admixed mineral content.

Table 4.13. Compilation of the principal suspended micro-organisms in natural and tailings drainage waters - Elliot Lake region.

	Sampling Station	Types of algae
September 1981	c	Ulothrix sp.
	E	Spirogyra sp.
	F	Spirogyra sp. Zygnenematales sp.
	G	Ulothrix sp.
	L	Phaeaster sp.
	J	Euglena elastica
	n	Ulothrix sp. Klebsormidium sp.
May 1982	c	Spirogyra sp. Ulothrix sp. Zygnema sp. Oedogonium sp. Tabellaria sp. Vaucheria sp. Rhizoclonium sp. Cladophora sp.
	K	Euglena elastica Diatoms
September 1982	P	Euglena elastica
	c	Spirogyra sp.
	G	Euglena elastica
	v	Ulothrix sp. Spirogyra sp.

Sampling stations in lower case are natural waters. Those in capital letters are tailings waters. See Fig. 4.1 for locations.

in the cells.

Uranium abundances, and concentration factors for Euglena communities are in the same range as these recorded for suspended particulates in tailings waters (Figs. 4.6, 4.7). Concentration factors for these two groups in tailings waters are comparable to those estimated for filamentous and unicellular algae suspended in natural drainage waters (Fig. 4.7). However, the absolute uranium abundance in tailings organisms is about one hundred times that of their counterparts in natural waters (Fig. 4.6). This observation is interpreted in termds of higher uptake where the aqueous uranium supply is also elevated (Fig. 4.6).

4.6 Multielement analyses of waters and micro-organisms

Comprehensive chemical analyses of waters, specifically of tailings discharge, has been conducted in the Elliot Lake region, with the principal objective of monitoring dissolved contaminants and their dispersion (Moffett and Teblier, 1978; Cherry, 1982; Blair et al., 1980). However little attention has been directed at the interaction of the biological domain with such heavy metal contaminated waters, excepting the immediate problem of tailings surface restoration (Murray and Moffett, 1977).

Given the magnitude of uranium uptake from tailings waters by Euglena, as described above, a selected number of multielement analyses were performed on both natural and

tailings waters, as well as the microorganisms of these two aquatic environments. If Euglena accepted up to 2.7 percent uranium by weight, what other dissolved chemical elements were also present in the algal cells, or what natural inorganic elements were displaced? Stations a and c on Pardee and Stintson Lakes respectively, upstream of the Nordic Main tailings impoundment (Fig. 4.1) were selected for a 'natural' water environment, given both the known low levels of dissolved uranium at these sites (Table 4.1, Figs. 4.2-4.4) and the prolific filamentous algal growth at site c. Analyses were also conducted on filamentous algae in natural waters of the Serpent river (station v, Fig. 4.1), upstream of Dennison Mines. For the tailings environment, discharge waters from the Stanrock impoundment (station p, Fig. 4.1), along with Euglena from this location plus site N in the Nordic Main discharge were analysed.

Chemical analyses were made by inductively coupled plasma emission spectroscopy (ICP) on preconcentrated waters, and on aqueous analyates of algae, dried, weighed and digested as described in Appendix I. ICP was employed, first because 24 elements can be determined simultaneously on a single solution, and second, because of the low levels of detection for most elements (typically 1 ppb in solution).

Table 4.16. Solute abundances for specified elements in World and North American river waters, along with Elliot Lake river and tailings waters (all figures in ppb [ng/ml]).

Element	World[3]	N. America[3]	Elliot Lake[1]						
			A		C		P		Standpipe[6] piezometer groundwater
	median abundances	median abundances	abundance[2]	concentration factor[4]	abundance[2]	concentration factor[4]	abundance[2]	concentration factor[4]	
Ca	15,000	21,000	6,700	0.3	4,700	0.2	115,000	5.5	
Si	13,000	9,000	900	0.1	630	0.07	8,100	0.9	
Na	6,300	9,000	1,160	0.1	820	0.1	9,860	1.1	
Mg	4,100	5,000	1,130	0.2	882	0.2	21,800	4.4	
K	2,300	1,400	610	0.4	600	0.4	6,960	5.0	
U	300 670 483		40	0.1	150	0.3	556,000	1,200	≤6,100
Al	238 400 332		50	0.1	50	0.1	35,600	107	
Sr	60 70 62		25	0.4	20	0.02	166	2.7	
Ba	45 20 27		12	0.4	13	0.5	6	0.3	
Zn	5.0 45 21		<2	<0.1	<2	<0.1	1,840	88	≤11
P	19 20 19.5		<60	<3	<60	<3	500	26	
Mn	20 7 13		49	3.8	78	6.0	2,230	170	
Tl	8.6 3.0 5.8		<1	<0.2	<1	<0.2	489	84	
Ni	10 0.3 5.1		2	0.4	2	0.4	402	79	0.3 3.6

1. Elliot Lake sample sites - see Fig. 4.1
2. Solute abundances for Elliot Lake waters determined by ICP
3. Median values for World and North American river solute abundances taken from Holland (1978), and arranged in order of decreasing val Where several figures are quoted, they represent the extreme range of median values reported from diverse sources in the literature. Figures between lines represent the average of the median values reported by Holland (1978).
4. Concentration factor = abundance in Elliot Lake river or tailings waters divided by North American median abundance for Ca, Si, Na, and K; and referenced to the average of World median abundances reported by Holland (1978) [column 2] for the remaining elements. - No figures for these elements reported by Holland (1978).
5. World average river water uranium abundance from Bloch (1981).
6. Data from Blair et al (1980).

Table 4.14. Continued

Element	World[3]	N. America[3]	Elliot Lake[1]						
			A		C		P		Standpipe[6] piezometer groundwater
	median abundances	median abundances	abundance[2]	concentration factor[4]	abundance[2]	concentration factor[4]	abundance[2]	concentration factor[4]	
Pb	4.0 3.0 3.6		<5	<1.4	<5	<1.4	861	240	≤2
Cr	5.8 1.0 2.7		1.2	0.4	3.3	1.2	113	42	
V	1.0 0.9 1.0		<0.3	<0.3	<0.3	<0.3	3.2	3.2	
Mo	0.35 1.8 0.83		<30	<36	<30	<36	200	240	
Ag	0.09 0.3 0.23		<5	<22	<5	<22	<5	<22	
Co	0.19 0.1 0.14		<3	<21	<3	<21	772	5.510	≤16
Th	0.096 0.1 0.098		<6	<61	<6	<61	316	3.220	
Be	-		<0.1		<0.1		2.4		
Cd	-		<7		<7		15		
Cu	-		2.0		1.7		469		
Zr	-		<3		<3		134		
U[5]	0.6		0.05	0.01	0.08	0.13	50	83	10 - 500

1. Elliot Lake sample sites - see Fig. 4.1
2. Solute abundances for Elliot Lake waters determined by ICP
3. Median values for World and North American river solute abundances taken from Holland (1978), and arranged in order of decreasing values. Where several figures are quoted, they represent the extreme range of median values reported from diverse sources in the literature. Figures between lines represent the average of the median values reported by Holland (1978).
4. Concentration factor = abundance in Elliot Lake river or tailings waters divided by North American median abundance for Ca, Si, Na, Mg and K; and referenced to the average of World median abundances reported by Holland (1978) [column 2] for the remaining elemen - No figures for these elements reported by Holland (1978).
5. World average river water uranium abundance from Bloch (1981).
6. Data from Blair et al (1980).

4.6.1 Chemical composition of natural and tailings waters

Solute abundances for an array of elements in Elliot Lake 'natural river' as well as tailings waters, along with data for their concentrations in World and North American rivers are reported in Table 4.14.

Waters of the 'natural' drainage basin are relatively dilute solutions, with all of the major dissolved components at 0.1 to 0.5 of their average concentrations in World and North American rivers. The only exception is manganese, which, with an average solute abundance of 49 ppb (Table 4.14) is about four times more concentrated than the World figure for riverine manganese. Given the combination of coarse grained metamorphosed 'Shield' rocks, recent glaciation, and a cool climate under which conditions chemical weathering is relatively slow, these low solute abundances are as expected.

Tailings drainage waters at site P are profoundly more concentrated solutions with respect to nearly all chemical elements, as anticipated from their low pH which supports elevated concentrations of dissolved solids in solution. Tailings waters are dominated by Fe, Ca, Si, Na, Mg and K, presumably from the hydrolysis of feldspar and ferromagnesian minerals, along with the oxidation of pyrite. The above listed elements are present at a concentration of 1 to 5 times their world riverine abundances, excepting iron, which is 1000 fold enriched. Even the relatively

insoluble element aluminum is present at 36 ppm, about 100 times the World average riverine figure.

Of the trace elements in solution, all are present at about 100 times their dissolved levels in typical river water, with Co, Th and of coarse uranium about 1000 fold greater. Inasmuch as only one sample of tailings water was analysed, these results are taken as guidelines, rather than as representative of tailings waters.

Measured solute abundances in the effluent are comparable to or slightly greater than data reported by Blair et al. (1980) for groundwater recovered from standpipe piezometers.

4.6.2 Chemical composition of algae

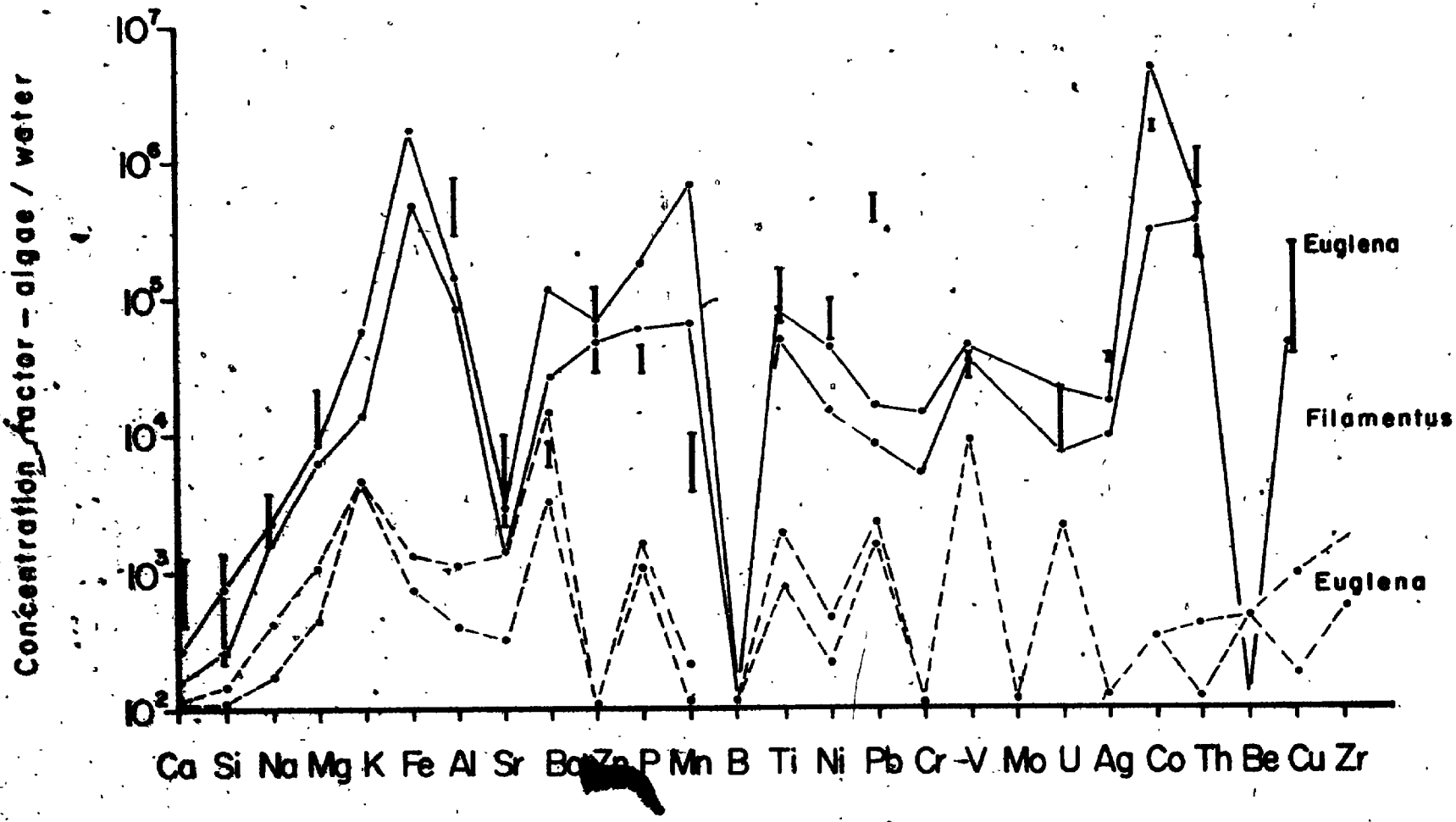
Filamentous algae growing in natural waters have high concentrations of Ca, K, Mg, and P, which along with C, N, S, H, and O that were not analysed for, are the essential chemical components of their cellular structures. Calcium accounts for 11 to 17 weight percent of the filamentous algae by dry weight, sodium 19 to 27, magnesium 7 to 9, potassium 8 to 35, and phosphorous 1.1 to 3.4 (Table 4.15). These values are comparable to the ranges of Ca, Na, Mg, and P for freshwater and marine microbiota (Trudinger, 1976; Chapman and Chapman, 1980; Spiedel and Agnew, 1982, p. 135, 140). However, they also contain spectacular concentrations of several other major and minor

Table 4.15 Abundances of selected elements in filamentous algae from natural waters, and Euglena from tailings waters. Elliot Lake.

Element	filamentous		Euglena		
	C†	V†	H†	P†	Q†
Ca ppm	107,900	175,000	2,340	7,060	8,350
Si	1,450	7,028	220	374	1,190
Na	190,900	271,300	-	1,700	4,340
Mg	95,300	68,200	9,290	19,800	24,500
K	80,700	345,200	32,200	28,000	32,100
Fe	193,000	698,700	401,100	579,700	761,200
Al	69,900	41,100	14,260	30,900	39,060
Sr	350	740	54.0	250	153
Ba	3,300	12,040	20.2	88	6.90
Zn	1,320	945	55.2	153	234
P	11,000	34,500	828	765	528
Mn	40,100	383,500	202	467	182
B	-	-	-	-	-
Ti	4,500	2,750	385	943	634
Ni ppb	270,000	822,000	83,000	110,500	182,000
Pb	540,000	288,000	1,290,000	1,740,000	1,910,000
Cr	108,000	288,000	-		
V	328,000	407,000	27,600	51,800	71,100
Mo	-		-		
Ag	35,000	20,000	-		7,000
Co	403,000	6,490,000	-		251,700
Th	322,000	411,000	18,400	76,500	113,000
Be	3,200	5,300	1,000		1,000
Cd	-		-		
Cu	793,000	840,000	73,600	173,000	414,000
Zn	134,000	239,000	64,400	119,000	208,000
U					

† for sample locations see Fig. 4.1.

Fig. 4.8. Concentration factors for specified elements in *Euglena* from tailings waters and filamentous algae from natural waters, Elliot Lake region. For *Euglena*, concentration factors referenced to composition of tailings waters in dashed lines, and to average world river waters in bars (see Table 4.14). Element arranged in order of decreasing abundance in average world river water (cf. Holland, 1978).


Concentration factor – algae / water
10^7
10^6
10^5
10^4
10^3
10^2
Euglena
Filamentus
Euglena
Ca Si Na Mg K Fe Al Sr Ba Zn P Mn B Ti Ni Pb Cr V Mo U Ag Co Th Be Cu Zr

dissolved species present in river waters, such as Fe (200,000-700,000 ppm), Na (200,000-27,000 ppm), Mn (40,000-380,000 ppm), Al (40,000-70,000 ppm), Si (1,400-7,000 ppm), Ni (270-820 ppm), Pb (540-290 ppm), Sr (350-740 ppm), Th (320-410 ppm), Ti (2,700-4,400 ppm), V (330-410 ppm), Zn (950-1,300 ppm), Ba (3,300-12,000 ppm), Co (400-6,500 ppm), and Cr (110-290 ppm). Even elements with exceedingly low dissolved concentrations in rivers (Table 4.14) such as silver (Ag=0.23 ppb) and zirconium (Zr<3 ppb) were detected in the filamenous algae where Ag = 20-35 ppm and Zr = 130-240 ppm.

Communities of Euglena thriving in tailings waters also have high concentrations of Ca, K, Mg and P - the essential chemical components of cellular structures. These elements are present at a somewhat lower level than in the filamentous algae, possibly a species specific effect. The Euglena contain much higher absolute abundances of many elements even than the filamentous algae discussed above, reflecting the elevated dissolved concentrations of these elements in the tailings waters. For instance the Euglena contain 40 to 70 wt % Fe, Al (14,000-40,000 ppm), Ba (7-90 ppm), Co (250 ppm), Cu (70-400 ppm), Mn (200-500 ppm), Ni (80-200 ppm), Pb (1,300-2,000 ppm), Si (200-2,000 ppm), Sr (50-200 ppm), Ti (400-900 ppm), V (30-70 ppm) and Zn (50-230 ppm) [Table 4.15]. Abundances as high as 70 wt. % may have significant errors. Elements

with low dissolved abundances such as Ag, Be, Th and Zr were also detected in Euglena, as follows: Ag (7 ppm), Be (1.0 ppm), Th (20-110 ppm) and Zr (60-210 ppm). The data for the algae are plotted in Fig. 4.8 referenced to the composition of their host waters. Much lower concentration factors in the former versus the latter plot illustrate the effect evident for U in Euglena (Fig. 4.7) that concentration factors diminish at progressively higher solute abundances. This may imply an approach to saturation of metal sites for the cells.

4.7 Summary and conclusions

The essential conclusions of these studies on the Elliot Lake region together with microorganisms of its aquatic environment are as follows:

1. Levels of dissolved uranium in natural drainage waters of the Elliot Lake district average 0.23 ± 0.19 ppb 1σ. This figure is in accord with uranium contents of rivers draining the Canadian shield in general, but about a factor of three lower than the mean global riverine solute uranium of 0.6 ppb.
2. Dissolved uranium in tailings effluent averages 85 ± 62 ppb at the Nordic Main, 220 ± 230 ppb 1σ for Westarm and 273 ± 215 ppb 1σ for the Stanrock impoundment.
3. In waters of Elliot lake, some 6 km downstream of the tailings impoundments, the mean uranium solute concen-

tration of 4.9 ± 3.9 ppb 1σ, represents about twenty times the background level of 0.23 ppb for natural drainage.

4. Fifty kilometers downstream of the mining district, where the Serpent river debouches into Lake Huron, its waters carry 1.2 to 1.9 ppb dissolved uranium, a seven fold enrichment over its headwaters and twice the uranium content of the lake into which it flows (0.6 ppb). This uranium spike in the Serpent river is likely due to the unusually uraniferous Huronian sediments constituting part of its drainage basin, as well as to U contamination from tailings effluent discharged into the river system.
5. Suspended microorganisms, which are chiefly algae, in natural waters contain on average 19,300 ppb U, and up to 1,200,000 ppb, the latter figure representing 1.2% of the cells by dry weight.
6. The average mass of water-borne suspended particulates (chiefly algae) was 11.2 ± 21.3 1σ mg/litre, and this component carries 216 ng U/litre, or about 45% of the total riverine uranium flux of the drainage system.
7. The partition coefficient, or average partitioning of uranium between microorganisms and waters of the drainage system, is 8×10^4, close to that of 2×10^4 for Thames River waters.
8. Growths of filamentous algae in natural waters contain

on average 4,500 ppb U, a factor of five less than the communities of suspended microorganisms. This may either be a species specific phenomenon, and/or a function of differences in the surface area/volume ratio of filamentous versus unicellular algae. A rod (filament) has a higher surface area/volume than a spherical unicellular shape.

9. Suspended microorganisms in tailings effluent have on average 184,000 ppb U ($\pm$ 358,000 1σ), about 10^3 to 10^4 times that of their aquatic habitat. Levels up to ~2,700,000 ppb were recorded.
10. The algae Euglena is the dominant microorganism in tailings waters, flourishing despite the low pH and contamination of many apparently toxic metals.
11. For the Elliot Lake region as a whole, there is a broad correlation of uranium abundance in microorganisms with dissolved uranium in their host waters. A general antivariance exists between concentration factor (U algae/U H_2O) and dissolved uranium.
12. Algae growing in both natural and tailings waters contain high concentrations of many elements in addition to U, including Ag, Al, Ba, Be, Co, Cr, Cu, Fe, Mn, Ni, Pb, Si, Sr, Th, Ti, V, Zn and Zr. Of these trace elements Fe, Ba, Zn, Mn, Ti, V, Ni, Pb, Cr, Ag, Co and Cu have concentration factors in algae of 10^4 to 10^6 times their aqueous abundances.

CHAPTER 5

THE CHEMICAL COMPOSITION OF ALGAE FROM SELECTED ENVIRONMENTS

5,1 Introduction

Data for the trace element composition of algae are sparse, and the number of elements reported is limited (cf. Trudinger, 1976). For this reason a reconaissance study was undertaken of selected marine and freshwater algae to determine their trace element contents, and to provide a comparison for the algae analysed from Elliot Lake. These data are compared to results obtained for Euglena and filamentous algae from Elliot Lake as detailed in chapter 6.

5.2. Materials and methods

For the marine environment, algae were obtained from the Atlantic coast of Canada, at Nova Scotia (Fig. 5.1), from the Pacific coast of Canada near Vancouver (Fig. 5.2), and from the Pacific in Australia. Freshwater algae were collected at Beauty Creek in the Canadian Rocky Mountains (Fig. 5.2), and from a continental saline lake (Lake Toxcoco) in Mexico. An example of prolific growth of thermophillic algae and bacteria from the hot spring area of Beowawe, Nevada, was also included. Those sites were selected to encompass a range of terrestrial environments, as well as to provide monocultures of specified algae. Details are reported in Table 5.1.

Algae were dried, weighed and then digested in refluxed boiling HNO_3. Solutions were further acidified with HCl, and taken to an appropriate volume, as described in Appendix I. No ashing of dried algae was employed, given the notorious effect of this procedure on loss of metals from the residue by volatilization. Solutions were analysed by inductively coupled plasma emission spectroscopy. This technique combines low levels of detection (typically 1 ppb) with simultaneous analysis of 26 elements.

5.3. Results

Abundances of 26 elements, along with uranium, for

algae from the three marine environments are reported in Table 5.2. Calcium, Na, Mg, K, and P are present in the highest abundances; these elements, along with C, N, S, H and oxygen that were not analyzed for, are the essential chemical components of cellular structure (Trudinger, 1976; Speidel and Agnew, 1983; Chapman and Chapman, 1980). Calcium accounts for 0.04 to 1.1 weight percent, Na for 0.6 to 5.1 wt. %, Mg 0.3 to 1.1 wt. %, K for 1.1 to 6.5 wt. %, and P for 0.06 to 0.25 wt. %. These figures are all within the range of values for Ca, Na, Mg, K and P in marine microbiota quoted by Trudinger (1976), but lower by a factor of 5 to 10 compared to their abundances in algae from Elliot Lake. This may be a species specific phenomenon.

Other elements which are present at significant levels include Fe (40-680 ppm), Al (56-430 ppm), Sr (210-800 ppm), Mn (25-110 ppm) and B (9-130 ppm). Several elements present at ppb or sub ppb levels in seawater have ppm contents in the algae: these include Ti, Ni, Pb, Cr, V, Ag, Co, Th and Cu. Uranium is present in the algae over a wide span of abundances, from 18 to 600 ppb. Zr, Cd, Be and Mo are at levels noticably low or below detection.

Concentration factors for elements in algae, referenced to their abundance in average marine water are plotted in Fig. 5.3. Excepting those primary components of the cellular structure, the most pronounced enrichments of 10^4

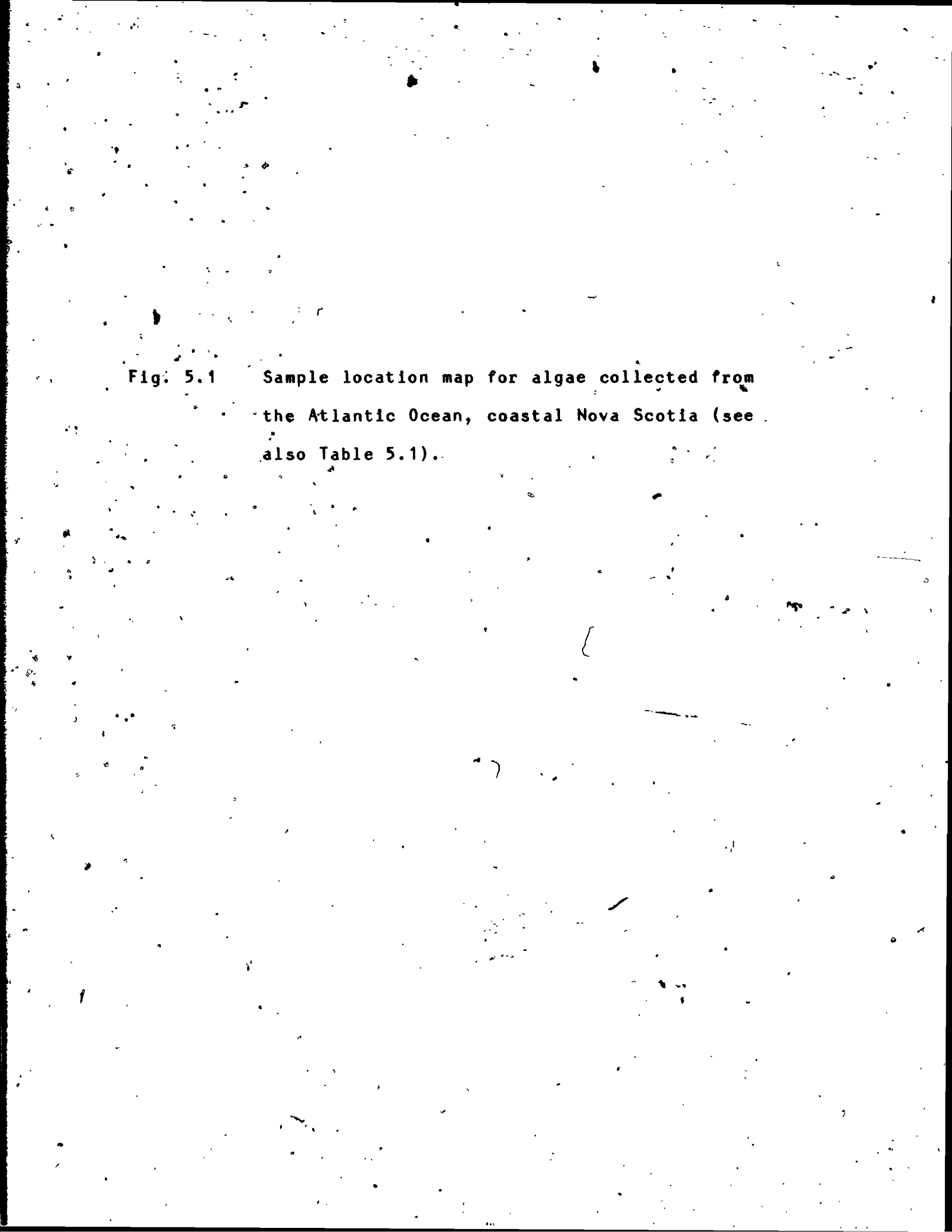


Fig. 5.1 Sample location map for algae collected from the Atlantic Ocean, coastal Nova Scotia (see also Table 5.1).

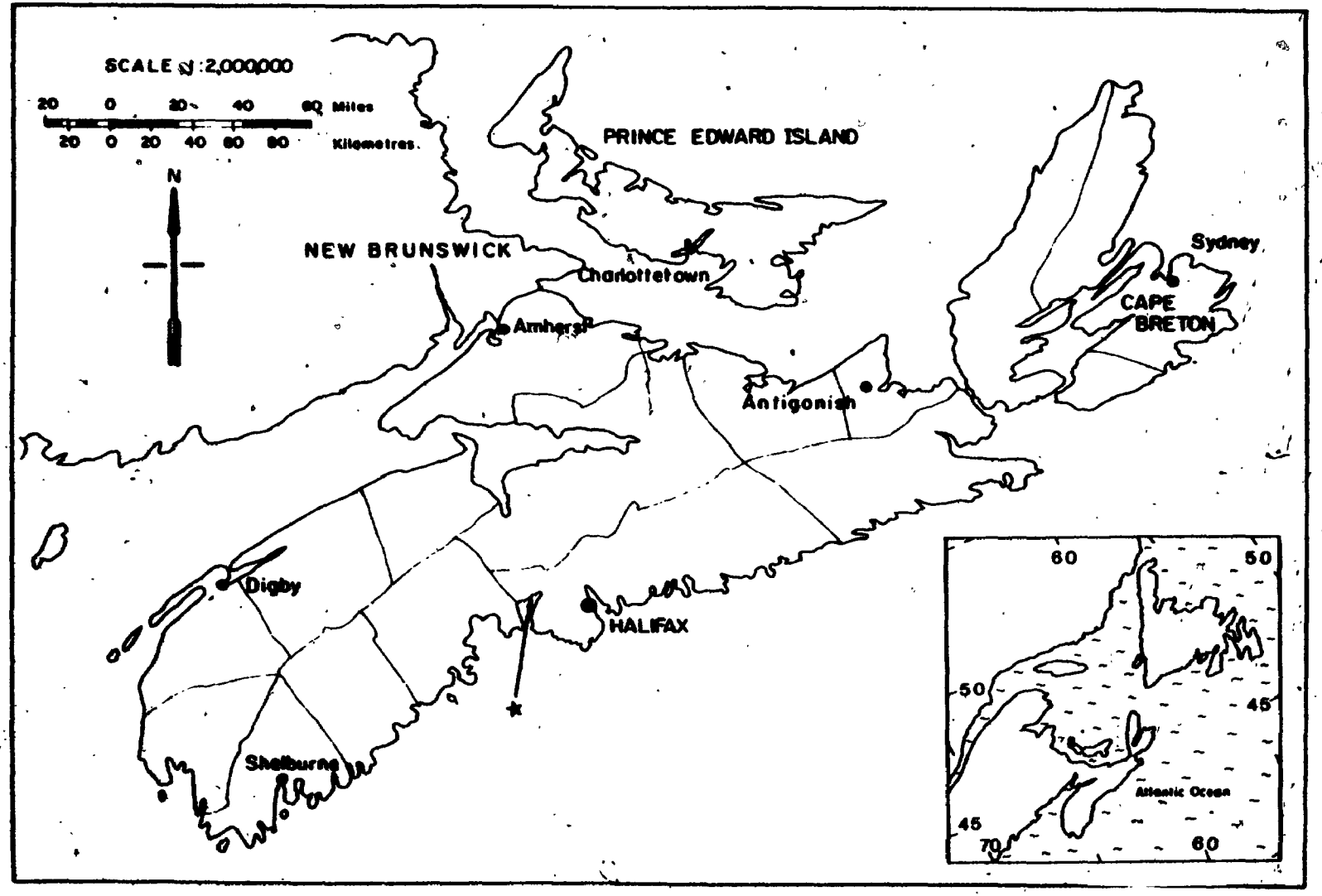

SCALE 1:2,000,000
20 0 20 40 60 Miles
20 0 20 40 60 80 Kilometres
N
PRINCE EDWARD ISLAND
NEW BRUNSWICK
Charlottetown
Sydney
CAPE BRETON
Amherst
Antigonish
Digby
HALIFAX
Shelburne
60
50
50
45
Atlantic Ocean
45
70
60

Fig. 5.2 Sample location map for marine algae collected in the vicinity of Vancouver, and freshwater algae obtained from Beauty Creek, near Jasper (see also Table 5.1).

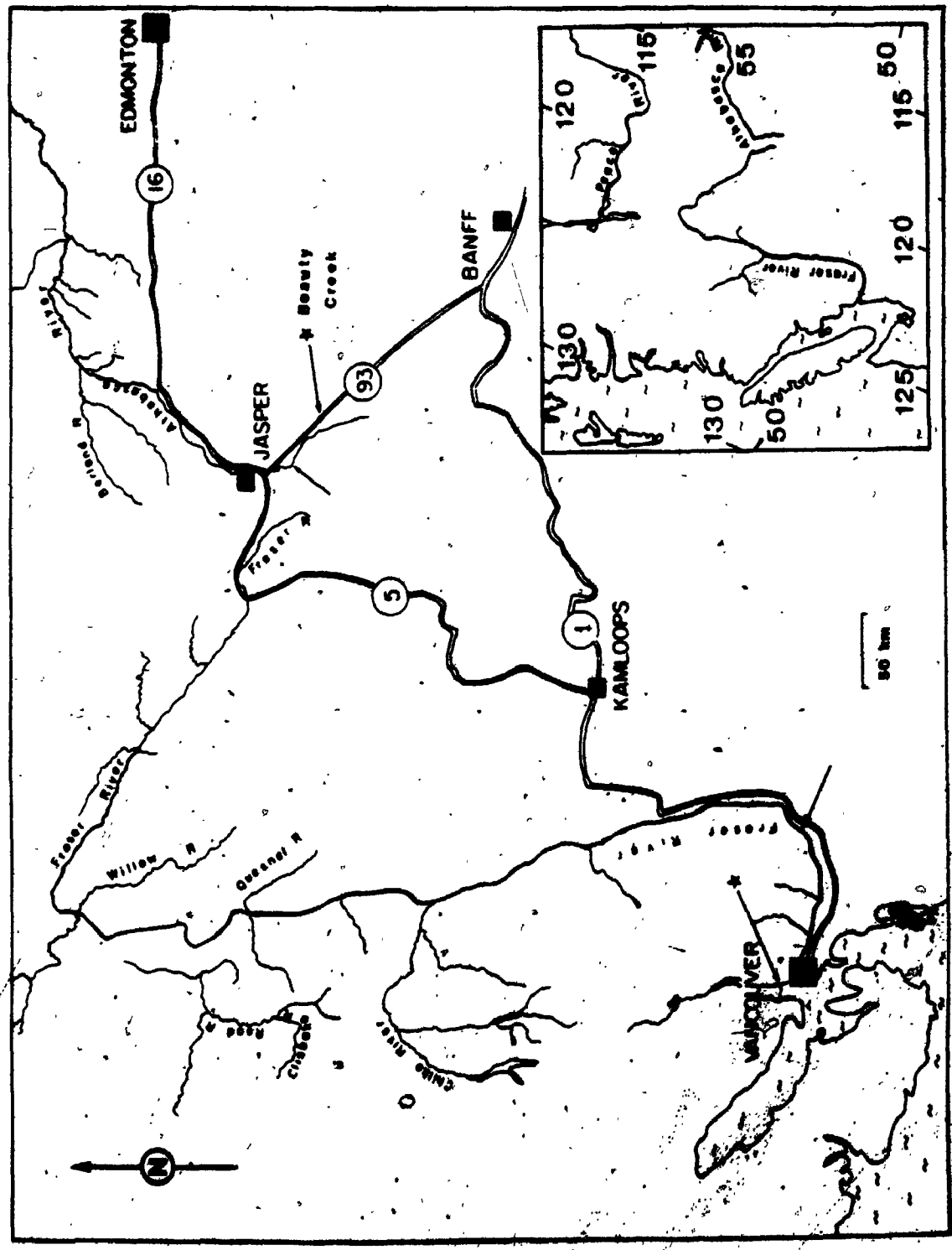

EDMONTON
16
JASPER
93
BANFF
Beauty Creek
Fraser River
5
1
KAMLOOPS
Willow R
Quesnel R
Chilcotin River
VANCOUVER
50 km
N
120
115
55
50
130
125

Table 5.3. Abundances of specified elements in selected freshwater and "hydrothermal" algae.

Element		Mexican†	Beowawe†	Beauty Creek†
Ca	ppm	767	10,660	45,800
Si		12.4	822	44,300
Na		7,562	35,200	-
Mg		1,597	15,350	15,222
K		7,455	14,350	21,887
Fe		218	39,200	2,223
Al		22.0	36,200	2,503
Sr		8.7	397	265
Ba		4.8	779	19.7
Zn		11.4	270	58.9
P		3,798	1,620	284
Mn		10.6	830	81.7
B		9.50	-	6.22
Ti		1.30	522	6.50
Ni	ppb	6,400	39,000	14,620
Pb		350	57,000	7,052
Cr		800	43,000	9,460
V			90,000	1,290
Mo				
Ag			4,800	
Co			83,000	
Th			48,000	4,300
Be			28,000	86
Cd				
Cu		210	406,000	4,100
Zr			13,000	
U		18.5	1,000	92

† locations, along with species of algae reported in Table 5.1, and figures 5.1-5.2.

Elements arranged according to abundance in average river water (Holland, 1978).

to 10^5 are for Fe, Al, Sr, Zn, Mn, Ti, Ni, Pb, Cr, Ag, Co and Th. Thorium concentration factors, which exceed 10^7 for several of the algae analysed, are so dramatic as to warrant cross-checking of the results by a more precise analytical technique such as instrumental neutron activation. Of the elements present at low absolute abundances, namely Zr, Cd, Be, and Mo, these also have low concentration factors, as is also the case for Si, Ba, B and U (Fig. 5.3). Overall, the calculated concentration factors are similar to those reported by Trudinger (1976) for marine microbiota, excepting K, Al, Ni, Ag and Co which are larger, and Pb which is smaller than his figures.

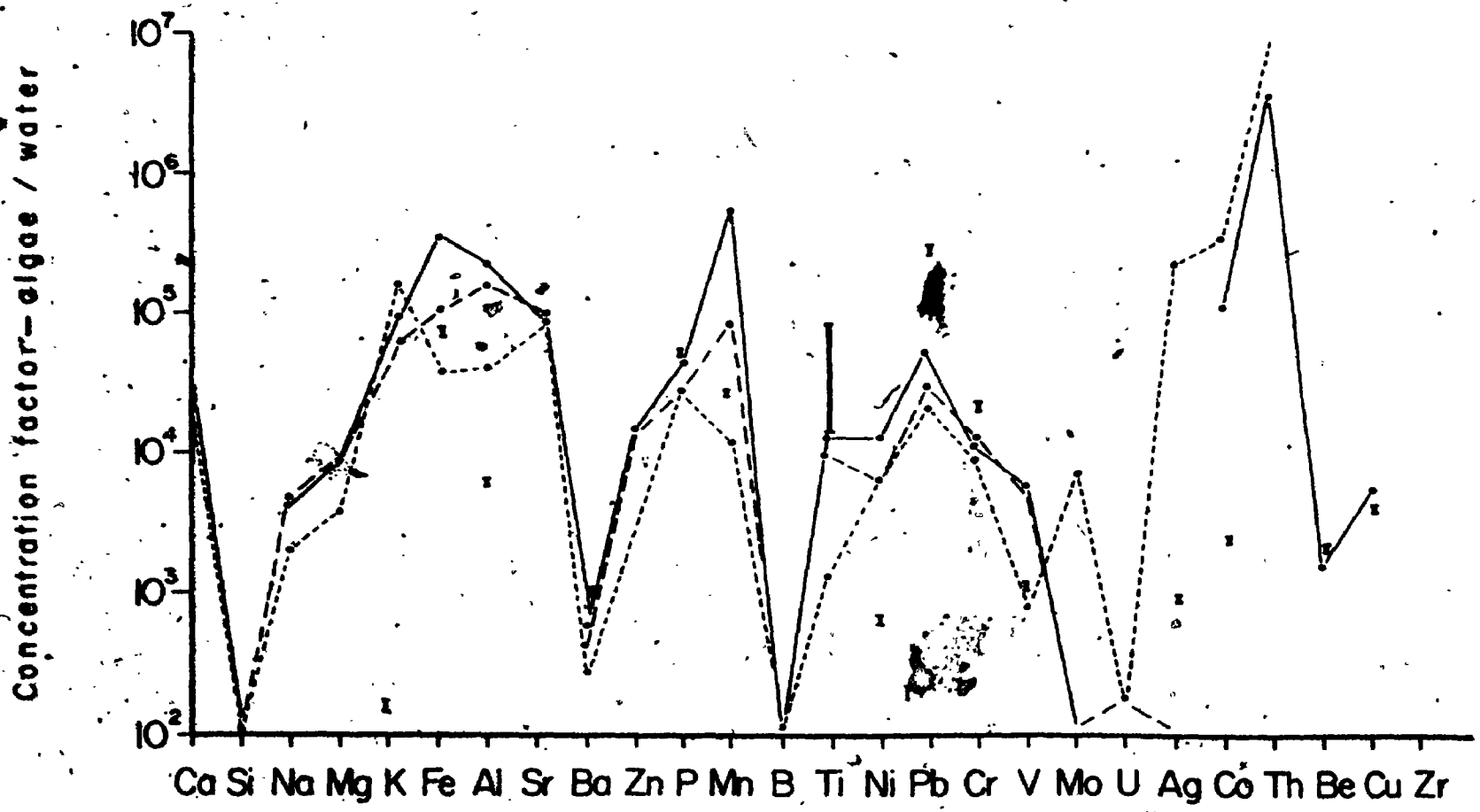
Data for algae from a saline lake (Lake Texcoco, Mexico), hydrothermal (Beowawe, Nevada) and freshwater (Beauty Creek, Alberta) environments are reported in Table 5.3. The chemical composition of these algae are dominated by Ca, Na, Mg, K and P (excepting O, N, H, S and O) as is the case for marine algae discussed above. Calcium accounts for 0.08 to 4.6 weight percent, Na 0.8 to 3.5 wt. %, Mg 0.15 to 1.5 wt. %, K 0.7 to 2.2 wt. % and P 0.03 to 0.38 wt. %. Overall these figures are comparable to the Ca, Na, Mg, K and P contents of the marine algae, and are within quoted ranges of values for these elements in microbiota (Trudinger, 1976; Speidel and Agnew, 1983). It is interesting to note that the golden filamentous algae from Beauty Creek (see Table 5.1) are the only ones analyzed

Table 5.2. Abundances of specified elements in selected marine algae, determined by inductively coupled plasma emission (ICP).

Element		Nova Scotia†			Vancouver†			S. Australia†		
		1	2	3	1	2	3	1	2	3
Ca	ppm	6,222	11,716	7,280	5,015	9,537	12,530	1,780	3,360	450
Si		31.1	36.5	<6	61.2	107	181	19.4	8.4	76.6
Na		44,350	51,272	20,400	6,120	11,900	19,762	38,500	42,484	38,434
Mg		10,550	11,252	4,846	3,400	7,514	10,858	7,760	9,954	9,220
K		34,800	23,600	64,450	11,560	21,930	28,481	29,970	32,600	65,152
Fe		683.2	205	73.8	134	136	111	49.5	39.8	220
Al		429	309	77.5	78.2	107	117	63.0	56.0	141
Sr		671	789	536.3	282	558	799	247	206	709
Ba		11.6	8.2	5.4	9.86	24.5	28.8	1.94	0.47	77.7
Zn		43.9	44.1	9.8	18.7	32.3	97.4	91.7	99.8	50.4
P		2,537	1,624	1,658	1,088	2,176	1,797	596	703	1,489
Mn		109	16.8	2.5	8.5	-	60.9	23.3	22.7	-
B		134	108	35.9	9.35	45.6	134	91.2	76.8	126
Ti		12.3	8.8	2.7	3.57	6	3.70	1.07	0.52	3.28
Ni	ppb	6,100	3,480	-	-	-	7,900	5,330	3,950	-
Pb		1,525	1,160	615	3,400	5,100	1,162	1,940	1,729	6,570
Cr		3,050	3,500	2,460	5,100	5,100	3,720	5,820	3,460	8,760
V		3,840	1,680	1,722	1,700	1,870	976	970	1,260	1,750
Mo		-	-	73,800	-	-	-	-		
Ag		-	-	1,700	7,480	1,190	-	-	395	
Co		4,600	-	15,900	15,300	13,600	3,250	-	3,950	
Th		5,490	4,640	12,300	3,400	15,300	-	3,880	2,700	2,190
Be		80	-	-	-	-	-	-	-	
Cd		-	-	-	-	-	-	-	-	
Cu		1,460	1,330	3,075	2,380	3,230	16,270	4,800	4,800	569,000
Zr		-	-	-	-	3,400	-	-		
U		18.3	464	566	85		84	131	198	395

† locations, along with species of algae reported in Table 5.1, and figures 5.1 - 5.2.
Elements arranged according to their abundance in average river water (Holland, 1978)

Fig.5.3. Concentration factors for specified elements in marine algae, referenced to their abundance in average marine water (cf. Holland, 1978). Short dashed lines, algae from Vancouver; solid lines, algae from Nova Scotia; long dashed lines, algae from S. Australia. Bars, data from Trudinger (1976) for marine microbiota (for details see Table 5.1). Elements arranged in order of their abundance in average river water (Holland, 1978).


Concentration factor – algae / water
10^7
10^6
10^5
10^4
10^3
10^2
Ca Si Na Mg K Fe Al Sr Ba Zn P Mn B Ti Ni Pb Cr V Mo U Ag Co Th Be Cu Zr

that have no detectable sodium (Table 5.3).

Other elements which are present at variable to high abundances are Si (12-44,000 ppm), Fe (220-39,000 ppm), Al (22-31,000 ppm), Sr (9-400 ppm), and Ba (5-780 ppm). For the Spirulina from Lake Toxcoco, and the algae from Beauty Creek, Zn, Ti, Ni, Pb, Cr and Cu are present at low ppm to high ppb levels. Spirulina are noticeable for the absence of V, Mo, Ag, Co, Th, Be, Cd and Zr above detection levels, and of these elements, Mo, Co, Ag, Cd and Zn are also below detection in algae from Beauty Creek.

Spectacular abundances of many metals are present in the thermophillic algae from Beowawe. Most pronounced are Fe (39,000 ppm), Al (36,000 ppm), Ba (780 ppm), Cu (410 ppm), Zn (270 ppm), Mn (830 ppm), Ti (520 ppm), Ni (39 ppm), Cr (43 ppm), V (90 ppm), Ag (4.8 ppm), Co (83 ppm), Th (48 ppm) and Zr (13 ppm). Most of these are elements typically enriched in the discharge waters of hot springs (cf. White, 1981).

Concentration factors for elements in algae, referenced to their abundances in world river water (Table 4.14) are plotted in Fig. 5.4. Patterns of enrichment are more scattered than is the case for marine algae (Fig. 5.3). Excepting those essential components of the cellular structure, the most pronounced enrichments of 10^3 to 10^4 are for Mn, Ni, Th and Cu. Algae from these three environments mimic the marine algae in having markedly low concen-

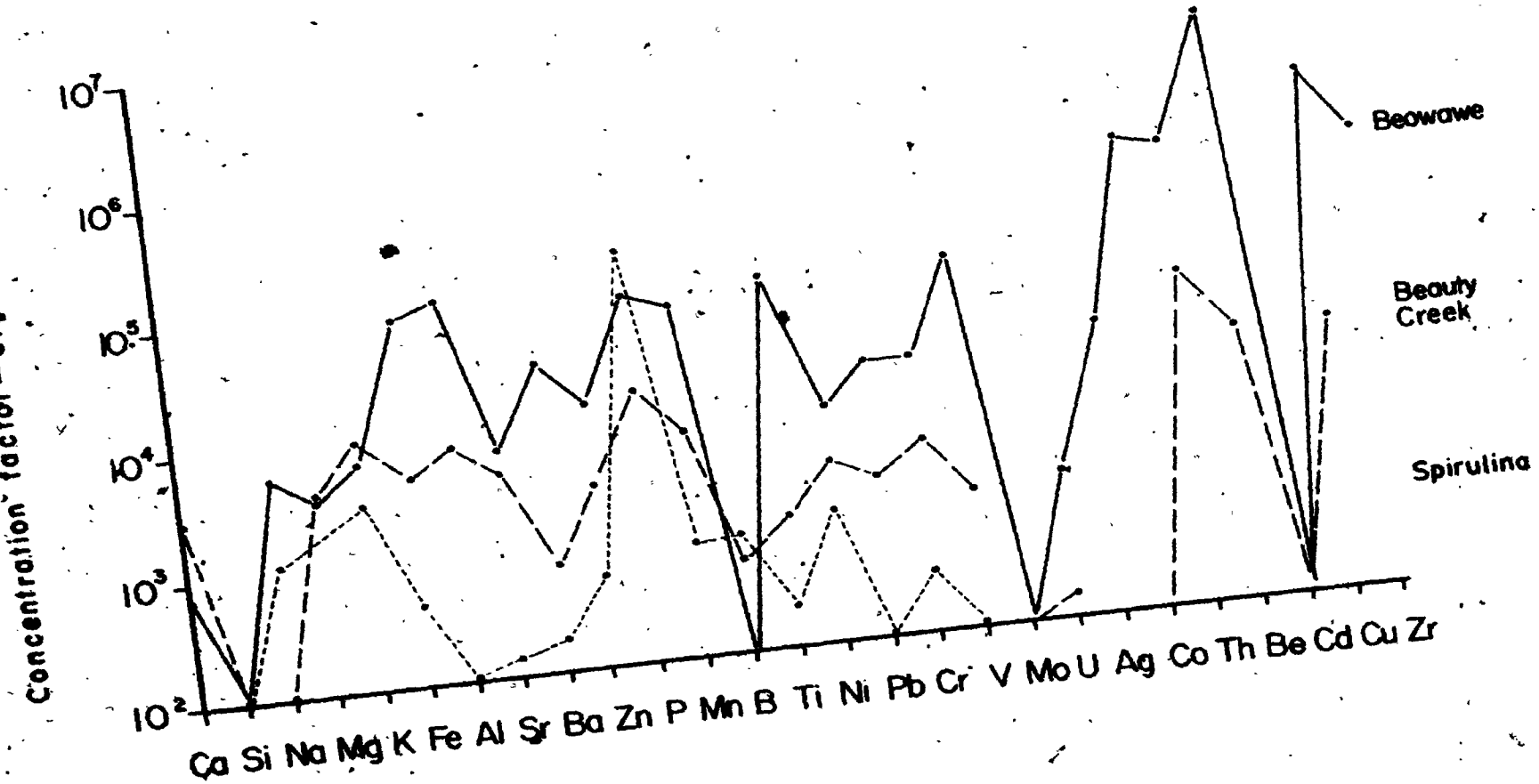
Table 5.3. Abundances of specified elements in selected freshwater and "hydrothermal" algae.

Element	Mexican†	Beowawe†	Beauty Creek†
Ca ppm	767	10,660	45,800
Si	12.4	822	44,300
Na	7,562	35,200	-
Mg	1,597	15,350	15,222
K	7,455	14,350	21,887
Fe	218	39,200	2,223
Al	22.0	36,200	2,503
Sr	8.7	397	265
Ba	4.8	779	19.7
Zn	11.4	270	58.9
P	3,798	1,620	284
Mn	10.6	830	81.7
B	9.50	-	6.22
Ti	1.30	522	6.50
Ni ppb	6,400	39,000	14,620
Pb	350	57,000	7,052
Cr	800	43,000	9,460
V		90,000	1,290
Mo			
Ag		4,800	
Co		83,000	
Th		48,000	4,300
Be		28,000	86
Cd			
Cu	210	406,000	4,100
Zr		13,000	
U	18.5	1,000	92

† locations, along with species of algae reported in Table 5.1, and figures 5.1-5.2.

Elements arranged according to abundance in average river water (Holland, 1978).

Fig. 5.4. Concentration factors for specified elements in algae from freshwater (Beauty Creek, Alberta), the saline lake Toxcoco (Spirulina) and hydrothermal (Beowawe, Nevada) environments. All concentration factors referenced to abundances of elements in average world river water (cf. Holland, 1978; Table 4.14), and arranged according to decrease of abundance.


Concentration factor — algae / water
10^7
10^6
10^5
10^4
10^3
10^2
Ca Si Na Mg K Fe Al Sr Ba Zn P Mn B Ti Ni Pb Cr V Mo U Ag Co Th Be Cd Cu Zr
Beowawe
Beauty Creek
Spirulina

tration factors for Si, B, Mo, and Cd. In common with the marine algae, they have pronounced enrichments of thorium, from 5×10^4 to 5×10^5 (Fig. 5.4).

It is interesting to note that the freshwater algae have on average much higher Fe (770-2,700 ppm), and up to 700,000 ppm Fe for filamentous algae at Elliot Lake, than the marine algae which contain an average of 180 $\pm$ 190 1 ppm Fe. It is important to emphasize that although the ICP analytical technique is linear over many orders of magnitude from the ppb to the low weight percent level (10,000 ppm), concentrations as high as 700,000 ppm (70 wt. %) may be in error by a factor of two. Irrespective of this, the extreme iron concentrations determined for Euglena imply an iron mineral associated with these algae, and accordingly a SEM/TEM analysis for detection of the iron as well as uranium phases present are warranted. This may reflect the higher iron contents of river waters (483 ppb Table 4.14) relative to seawater which has 2 ppb Fe. Euglena from tailings waters at Elliot Lake possess 400,000 to 760,000 ppm Fe, and thermophillic algae at Beowawe 39,000 ppm Fe, both of which are thriving in waters with high relative iron solute abundances.

Uranium is present at widely ranging levels from 18 to 1,000 ppb, the highest figure being for the hot spring algae.

These results are discussed collectively with data from Elliot Lake in chapter 6.

CHAPTER 6

DISCUSSION AND CONCLUSIONS

6.1 Introduction

Sporadically elevated abundances of certain elements including Cu, Pb, Zn, Ag, As, Sb, Cr, V, Co, Ni, U and Th occur in organic rich sedimentary rocks such as coals, petroleum, and black shales (Turekian and Wedepohl, 1961; Vine and Tourtelot, 1970; Trudinger, 1976; Valcovic', 1978; Meloche, 1982; Speidel and Agnew, 1982). For instance, Vine and Tourtelot (1970) reported that of a large array of elements enriched in black shales relative to their crustal average, many, including Ag, Mo, Zn, Ni, Cu, Cr, V, Co, Pb, La, Y, Se, U and Th were specifically associated with the

organic fraction. There is a general concensus that such anomalies may reflect mediation of the biosphere between the hydrosphere and lithosphere, whereby the strong metal complexing character of biological molecules of cells, or the biomolecules of their degradation, is responsible for selective transfer of dissolved components from aqueous to sedimentary reservoirs (Trudinger, 1976; Fyfe, 1980; Meloche, 1982; Speidel and Agnew, 1982).

In view of the above, this chapter briefly reviews the role of certain trace elements in the biochemistry of living organisms. Chapter 6 continues with a brief synthesis of information on the trace element inventory of hydrocarbon rich rocks with specific reference to petroleum. In the final section, results for uranium in algae are synthesized collectively, and discussed in the context of global element cycling.

6.2 Macro and micronutrients

The bulk of living matter consists of eleven elements which have low atomic weight, these are H, C, N, O, Na, Mg, P, S, Cl, K and Ca. Along with the major elements, certain trace elements which include heavy metals are recognized as essential micronutrients for warm blooded animals: these are F, Si, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Mo, Sn and I (Valcovic', 1978; Mertz, 1981). In addition to the major chemical components of cellular structures, plus trace

element micronutrients, both single cell and metazoan organisms are known to induce indigenous ancilliary biomineralization. Examples include fluorite in mollusca, and iron oxides in the Monera, Protoctista, Fungi, Animalia and Plantae Kingdoms (Lowenstam, 1981; Degens, 1976). More bioessential and ancilliary chemical element constituents may yet be identified.

In the context of the above, algae, along with many other microorganisms, can concentrate trace elements out of natural waters far in excess of metabolic requirements (Trudinger, 1976). Concentration factors up to 60,000 for some elements in plankton and algae were measured by Bowen as early as 1966 (Bowen, 1966).

6.2.1 Biological role of trace elements

The great majority of trace elements serve chiefly as key components of enzyme systems and metabolically active pigments. Enzymes in which metals are tightly bonded are termed metallo-enzymes, and if the metal atom is removed the enzyme usually loses the capacity to function. Clinical studies of many trace element deficiencies have revealed pathological disorders, suggesting either that there are many trace element dependent enzymes, or that these elements participate in a manner as yet unknown in the activity of other compounds.

The biological role of many trace elements has been

documented by Valcovic' (1978), and Speidel and Agnew (1983, pp. 180-183). Below, only those metals utilised in the experimental studies are briefly reviewed from the two aforementioned sources.

Barium (Ba)

Barium resembles calcium chemically, both belonging to Group 2A of the periodic table. It occurs in plant and animal tissues in highly variable proportions, but the only conclusive evidence as yet for any essential function of Ba is that of $BaSO_4$ in certain marine organisms (Lowenstam, 1981). Soluble barium salts are skin and mucous irritants, and in animals BaO and $BaCO_3$ may induce paralysis. Ba at elevated levels is poisonous to most plants.

Cobalt (Co)

Cobalt is known to be an essential element for blue-green algae; it is also essential for some bacteria, fungi and green algae. There is no clear evidence at present of cobalt necessity to higher plants; values exceeding 1 ppm in plants are rare. Cobalt is unique among trace elements, being biologically active for the higher animals only when incorporated in vitamin B_{12} or as one of the cobalamide derivatives.

Nickel (Ni)

Nickel is an essential trace element widely distributed in very low concentrations in both plant and animal tissues. In plants it can be highly toxic depending on the exact Ni compound and abundance. The uptake and toxic effects of Ni to algae were discussed in chapter 2.

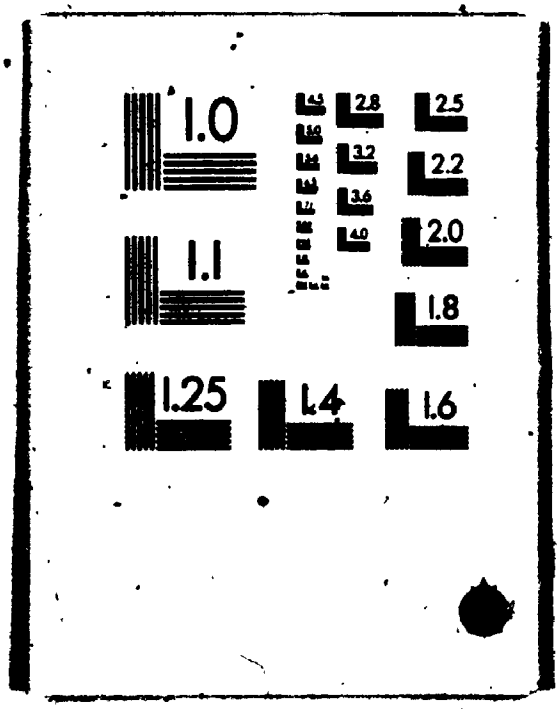
Vanadium

Vanadium is an essential element for some fungi and green algae. There is no clear evidence that vanadium is crucial for higher plants, although its presence in small quantities is thought to stimulate growth of higher green plants (Valcovic', 1978). At higher environmental levels, V is thought to partially substitute for Mo in NO_3 reductase. Vanadium is known to inhibit cholesterol synthesis in animal tissues, and specifically to counteract cholesterol synthesis catalysed by the presence of manganese. Indirect evidence of vanadium uptake by algae is known from the respiratory disease common to maintenance personell of oil fired burners, induced by V_2O_5 residues.

Cobalt, Ni and V are also distributed in bacteria in various metalloenzymes (e.g. Ni in Co dehydrogenase and Co in peroxidases).

4 4

OF / DE



6.3. Synthesis of results for algal compositions

In summary, all of the algae analysed, including those from Elliot Lake, have a composition dominated by Ca, Na, Mg, K and P, which along with C, N, S, H and O, are the essential chemical components of cellular structures.

High absolute abundances coupled with concentration factors of 10^4 to 10^5 were found for Fe, Al, Ba, Zn, Mn, Ti, Ni, Pb, Cr, V, U, Ag, Co, Th and Cu in Euglena and filamentous algae at Elliot Lake. For the marine algae Fe, Al, Sr, Zn, Mn, Ti, Ni, Pb, Cr, Ag, Co and Th are enriched by 10^4 to 10^5. Thermophillic algae in the Beowawe hot springs are characterized by concentration factors of 10^4 to 10^5 for Fe, Al, Ba, Mn, Ti, Ni, Pb, Cr, V, Ag, Co, Th, and Cu. Most of the algae analyzed have both low absolute abundances and low concentration factors for Si, B, Mo, Cd and Zr.

In general, algae from freshwater, tailings, and hydrothermal environments have significantly greater iron contents than their marine counterparts, probably reflecting the relatively lower marine aqueous Fe abundance.

6.4 The metal inventory of algal hydrocarbons

6.4.1 Introduction

It has long been established that in large part, crude oils are the product of degraded algal accumulations, and that such 'oils' contain a distinctive complement of heavy

metals including U, Ni and V. Changes in metal contents of algae from natural living communities through burial and diagenesis, to migration of matured oil into the reservoir rock may occur. However, to a first approximation petroleum hydrocarbons provide a record of the metals extracted by populations of microorganisms, chiefly algae, from natural fresh and marine waters. This section reviews the present data on heavy metals in crude oils.

6.4.2 Trace elements in crude oils

Besides hydrocarbons, compounds of sulphur, oxygen and nitrogen along with trace metals are minor constituents of crude oils. Metals, present as metallo-organic compounds are present in all crude oils to varying extents. Average trace element abundance data for petroleum hydrocarbons vary widely for crude oils from different parts of the world.

The most abundant metals are V (7.5-1,110 ppm), Ni (9.4-117 ppm), Fe (0.70-69 ppm), Co (13.5-0.003 ppm), and Hg (23.1-0.027 ppm) uranium reported at 0.015 ppm in Libyan crude oil (Valcovic', 1978).

An investigation of the uranium content of crude oils from the Western USA was undertaken by the U.S. Geological Survey in 1953, with results summarized by Vladovic' (1978). Uranium was found to occur in an oil-soluble form ranging in concentration from 0.1 to 13 ppb, with an

average of 1.4 ± 2.3 ppb. In ash fractions U contents were 45 to 200 ppm. Uranium contents of crude oils are generally independent of the type of reservoir rock. The humic acids indigenous to lignites, peats and other woody materials are thought to be the agents responsible for extraction of U from aqueous solutions. No secondary enrichment of uranium in oils would be expected by this mechanism, given the absence of humic acids in crude.

Average amounts of these metals in crude oils is much less than their crustal abundance (cf. Shaw, 1980), but greater by orders of magnitude than their levels in fresh or marine waters.

6.4.3 Chemical forms of metals in petroleum

Vanadium and nickel porphyrins were discovered in bitumens, coals and shales by Treibs (1935). Porphyrins are chelates having a closed planar ring structure, with the chelated metal held in the central area of the ring (Fig. 6.1). Hemoglobin and chlorophyll derivatives are typically associated with porphyrins in crude oil, forming the basins for the well known association between chlorphyll in plants and the process of petroleum formation (Valcovic', 1978). Because some of the metals present in crude oil appear in distillates, the compounds are presumably volatile, to some degree.

According to Yen (1975) the metallic compounds in

petroleum may be classified as follows:

(a) Metalloporphyrin chelates (V, Ni).

(b) Transition metal complexes of tetradentate mixed ligands (V, Ni, Fe, Cu, Co and Cr).

(c) Organometallic compounds of Hg, Sb, As (etc).

(d) Carboxylic acid salts of the polar functional groups of resins - which include those of Mo, Zn and Ge.

(e) Colonial minerals such as silica and NaCl.

Filby et al. (1975) separated crude oils into various fractions, and found that the ashphaltic component specifically, as well as the resins, contained the majority of the V, Ni, Co, Fe, Hg, Zn, Cr and Sb.

Metallo-organic complexes derived from biological precursors have been shown to occur in many types of sediments (Hunt, 1975). Yen (1975) suggests that magnesium removal from chlorphyll is accompanied by V and Ni introduction. Thus, although much of the metals in crude oils were probably indigenous to the algal precursors, some may have been acquired from pore waters. For instance, Filby (1975) suggests that the following pathways for incorporation of metals into crude oil may be relevant;

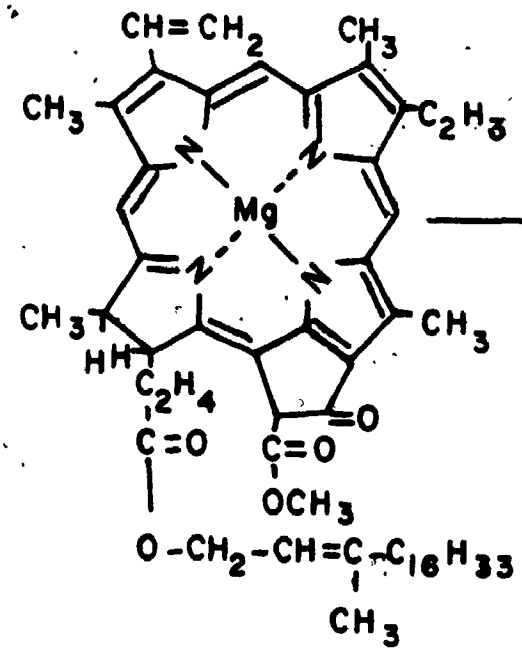
(a) through burial and diagenesis of metal complexes of the original biological material.

(b) transfer of metals from clays, or formation brines into the petroleum hydrocarbons.

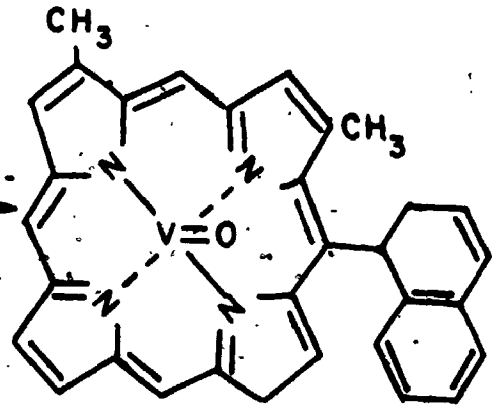
(c) and (d) through metal uptake from rocks or aqueous

Fig. 6.1 The origin of vanadium petroporphyrins by the removal of magnesium from chlorophylls and subsequent introduction of vanadium (modified after Yen, 1975).

CHLOROPHYLL a



ms-a-NAPHTHYLPORPHYRIN



phases either during migration, or in the reservoir rocks.

The first of these, the biological precursor, is thought to be the most important.

6.5 Uranium uptake by algae, a summary

Data for uranium contents of algae are collected in Fig. 6.1 for experiments of chapter 2, the Thames River, Elliot Lake region and miscellaneous algae discussed in section 5.1. Inspection of the summary diagram reveals a broad trend of increasing uranium contents of algae at progressively higher levels of dissolved uranium. In the quasi-natural habitat of Elliot Lake, where aqueous uranium is in the 100-200 ppb range, both suspended algae as well as monocultures of Euglena acquire on average 4×10^5 ppb U, and up to 2.7 percent by weight. Experiments were conducted at levels of up to 2 ppm, where the trend to still higher algal uranium concentrations is evident for Ankistrodesmus. Growth of Ankistrodesmus monocultures was almost completely depressed at 5 ppm dissolved U, but this certainly exceeds uranium levels likely to be encountered in either natural or tailings environment.

Experimental results for Ankistrodesmus conducted at much lower uranium solute abundances of 0.7 to 40 ppb mimic their natural counterparts in yielding lower algal uranium concentrations, but much higher concentration factors

Fig. 6.2. Summary diagram of uranium concentrations in various algae versus the dissolved uranium content of their aqueous medium. Numbers adjacent to bars or solid circles refer to the experimental series 1 through 6 reported in chapter 2.

a,b,c - marine algae, Nova Scotia

d,e - marine algae, Vancouver

f,g,h - marine algae, S. Australia

i - Spirulina, Lake Toxcoco, Mexico

j - thermophillic algae, Beowawe, Nevada

k - filamentous algae, Beauty Creek, Alberta

(for details see Table 5.1)

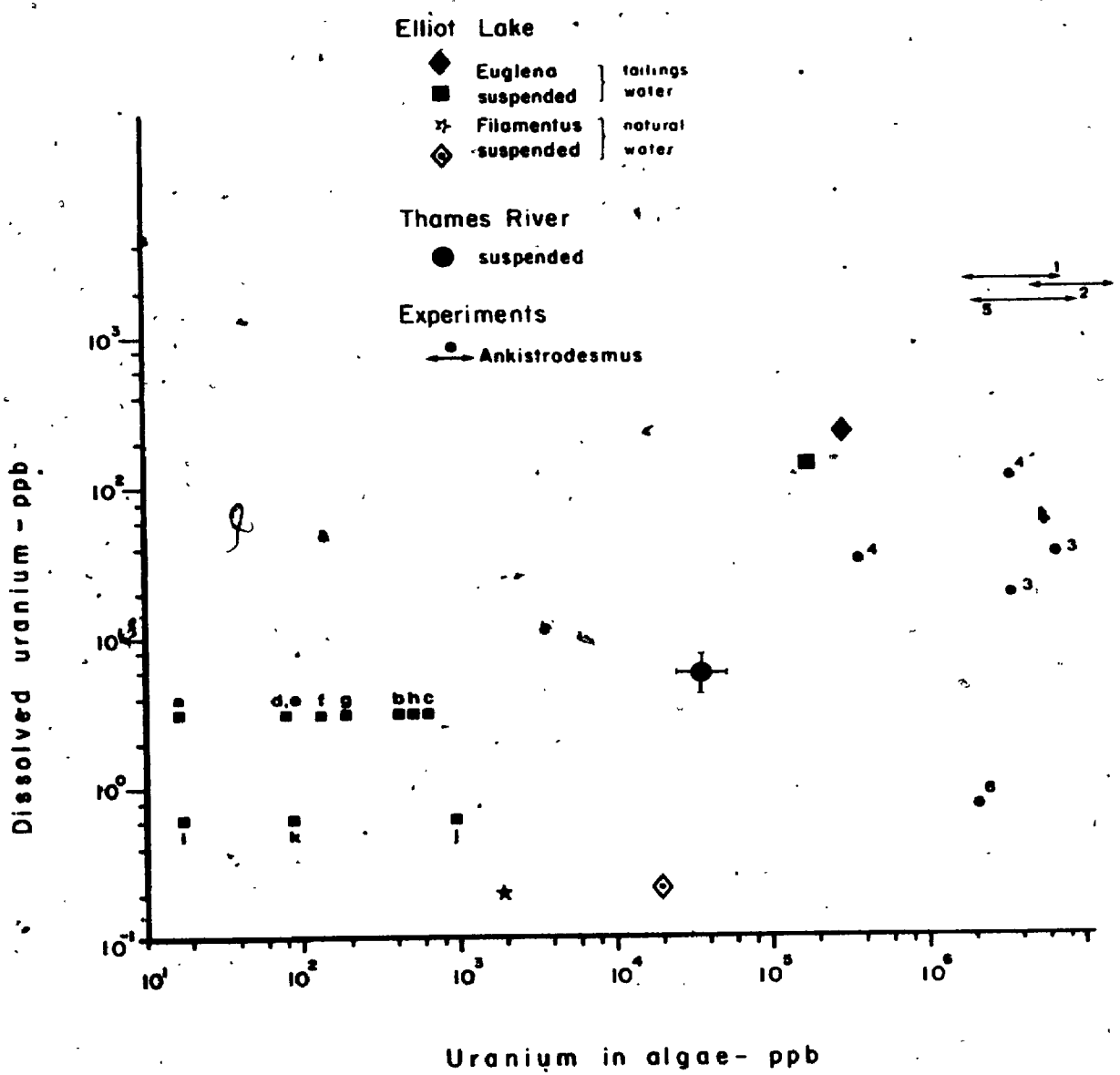

Elliot Lake
Euglena
suspended
tailings water
Filamentus
suspended
natural water
Thames River
suspended
Experiments
Ankistrodesmus
Dissolved uranium - ppb
Uranium in algae - ppb
bhc

Fig. 6.3. Summary diagram of concentration factors for various algae versus the dissolved uranium content of their aqueous medium. Numbers adjacent to bars or solid circles refer to the experimental series 1 through 6 reported in chapter 2.

a,b,c - marine algae, Nova Scotia

d,e - marine algae, Vancouver

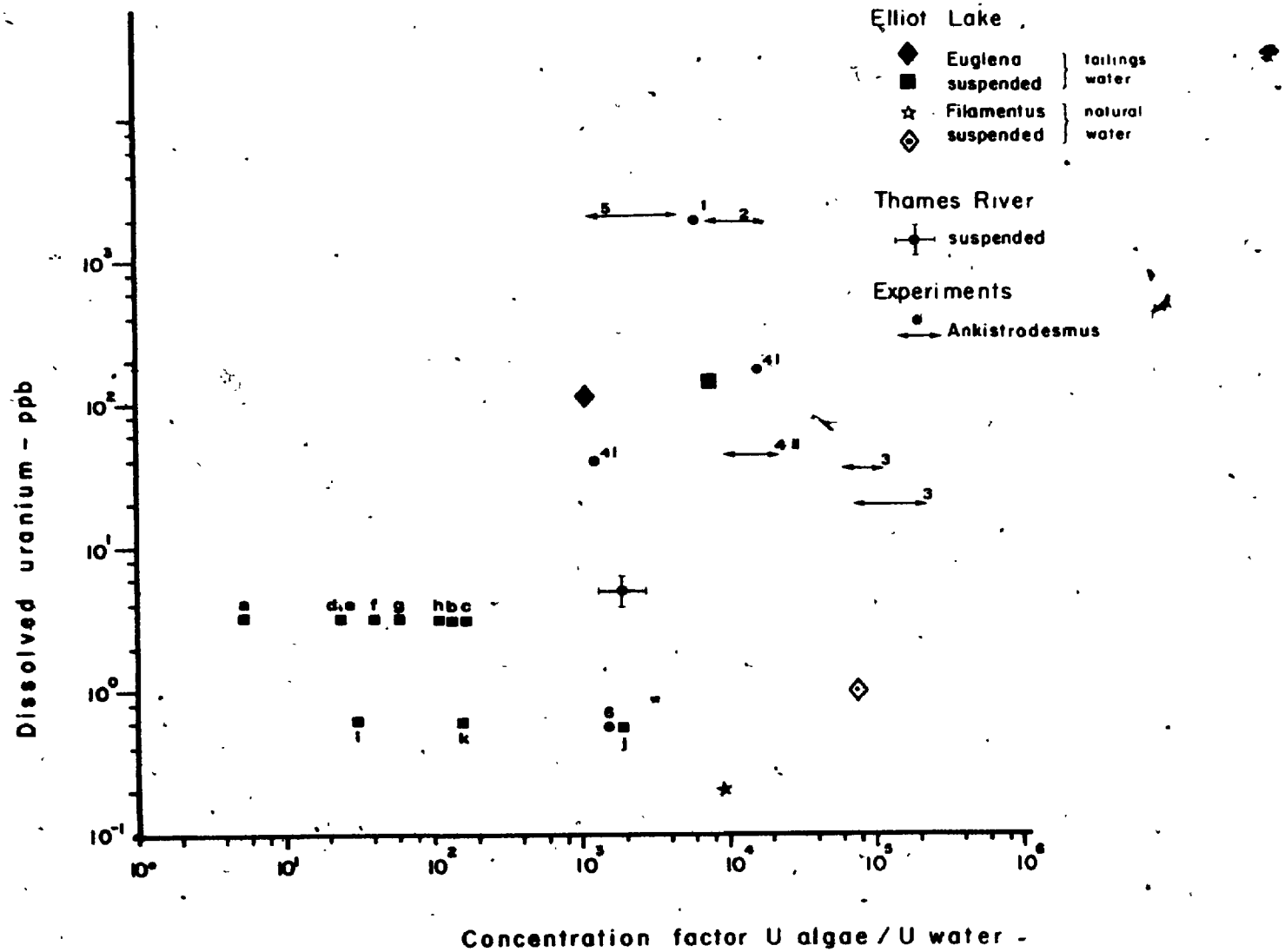
f,g,h - marine algae, S. Australia

i - Spirulina, Lake Toxcoco, Mexico

j - thermophillic algae, Beowawe, Nevada

k - filamentous algae, Beauty Creek, Alberta

(for details see Table 5.1)


Elliot Lake
Euglena suspended
tailings water
Filamentus suspended
natural water
Thames River
suspended
Experiments
Ankistrodesmus
Dissolved uranium - ppb
Concentration factor U algae / U water

(experiments 3, 4, 6, Figs. 6.2, 6.3). Results for the miscellaneous algae, a) through f), are somewhat scattered, but all reveal a significant capacity for uranium uptake in natural freshwater, hydrothermal and marine environments (Figs. 6.2, 6.3). Lower algal uranium levels in natural environments compared to the single metal spike experiments, even at low solute concentrations, almost certainly reflects the competition of other dissolved metals in natural waters, with U, for the algal metal sites.

Collectively, these results are interpreted as evidence for the role of microorganisms, specifically algae, in mediating the transfer of uranium from the hydrosphere to sedimentary rocks, of which several organic rich examples are known to be enriched in uranium (Degens, 1974; Degens et al., 1977; Fyfe, 1980; Nash et al., 1981). It is possible that up to 50% of the global river flux of dissolved uranium estimated at 1.92×10^{10} g/yr (Bloch, 1980) is extracted and sedimented by microorganisms. By extension, the experimentally determined uptake of Ba, Co, Ni and V, as well as the significant concentrations of Fe, Al, Ba, Zn, Mn, Ti, Ni, Pb, Cr, V, Ag, Co, Th and Cu in natural marine and freshwater algae may signify an important role of microorganisms in the geochemical cycling of these elements.

Appendix I

Analytical methods and calibration for the determination of uranium, barium, cobalt, nickel and vanadium in waters and algae

General statement

Both the laboratory experiments and natural materials collected during the course of these studies required the precise analysis of uranium at ppb to sub-ppb levels, involving only a few tens of nanograms of uranium. For instance a 1000 g aliquot of water with 0.05 ppb dissolved uranium contains only 50 ng of the metal, and 10 mg of algae with U at 1000 ppb contain a mere 10 ng U.

Two of the most sensitive analytical techniques for determination of U at low levels are fluorimetry and delayed neutron counting. The former has an absolute detection limit of 20 ng U, and the latter 40 ng. Given the low quantities of uranium encountered in these studies fluorometric analysis was employed throughout for both algae and waters: however a number of samples were also analysed for U by delayed neutron counting, in order to compare the methods, and to provide a cross check on the fluorimetric results. These techniques are described separately below.

Collection of waters and preconcentration

About two litres of water were collected for analysis of dissolved uranium at each sampling station for the Elliot Lake and Thomas river studies. The waters were placed in one litre polypropylene bottles, that had been precleaned with 50% HNO_3 and DIW, then acidified on site with premeasured aliquots of 10 ml reagent grade HNO_3.

In order to collect particulates separately for microscopic examination and determination of uranium, the two litre volumes of water were vacuum filtered through a 0.45 m (or 1.2 µm and then 0.45 µm filters, for samples with copius algae to speed filtration) type RA millipore filter paper. Volumes were measured precisely prior to filtration, and corrected for 20 ml added HNO_3, since some loss of water by evaporation is experienced from the Erlenmeyer flasks under vacuum conditions. Filtered waters were then evaporated to <25 ml in 1 litre teflon beakers at a temperature of 70°C, transferred to a 25 ml volumetric flask, acidified with 5 ml HNO_3 and taken to volume, giving a preconcentration of about eighty.

For those samples in which U was analysed by both fluorimetry and delayed neutron counting, the waters were preconcentrated to 100 ml, then split to 25 and 75 ml aliquots, the former for fluorimetry and the latter for delayed neutron counting. All glassware and teflon beakers were cleaned with 50% HNO_3 followed by DIW.

Acidification of waters on site, a universally adopted procedure, prevents loss of dissolved U by adsorbtion onto the container walls, and subsequently to the filtration equipment. The latter problem was examined in chapter 4, where it was demonstrated that for natural waters containing 0.5 ppb U, less than 20% of the total uranium was lost from the water onto glassware during filtration and much of this may have been indigenous to algae not the water; and for waters with greater than 10 ppm U

the absolute loss to equipment increases, but represents a smaller proportion of the total uranium present (Table 4.2).

One intractable problem is the unknown effect of acidifying natural waters on the uranium content of suspended particulates. Acidification probably acts to strip surface sited uranium from microorganisms and mineral particulates, decreasing their uranium inventory and thus artificially increasing that of the waters. The magnitude of this effect is not known, but it should be recalled that a few milligrams of suspended algae in some Thames river and Elliot Lake waters contained from 10% to in excess of half of the dissolved U present in 2000 g H_2O. An observation that bears on this problem comes from washing glassware with 50% HNO_3 after filtration of waters. The largest quantities of uranium recovered in this process were from unusually algal-rich waters, where an appreciable amount of dead algae had stuck to the glass reservoir as the water level fall during filtration, having resisted efforts to wash them off with DIW from a squeeze bottle. It is concluded that the uranium was derived from these algae, and hence that on site acidification does not digest most of the uranium indigenous to suspended algae.

Digestion of algae

Large populations of filamentous algae weighing 0.5 to 2 kg were gathered by hand in the field using surgical gloves to avoid possible contamination. The algae were washed in clean natural river water to remove attached particulates, placed in a

polythene bag and drained of excess water.

In the laboratory the algae were transferred to a preweighed 1 litre or 100 ml teflon beaker depending on the amount of algae present, and dried to constant weight at 65 ± 5°C for 70 hours, prior to final weighing.

Ashing of organic matter as a preconcentration process prior to analysis of metals is notorious for loss of the metals to the volatilized component. Thus all preconcentration of the algae was performed by digestion. Dry algae were initially digested in boiling nitric acid in a covered beaker, and finally in a mixture of boiling nitric and perchloric acids for three hours. The digestates were diluted with DIW and taken to appropriate volumes of 10, 25, 100 or 1000 ml, depending on the mass of algae involved. For some samples containing larger quantities of algae, the 100 ml volume was split into 25 and 75 ml aliquots, for analysis by fluorimetry and delayed neutron counting respectively.

Uranium analysis by neutron activation delayed neutron counting

Uranium analysis by neutron activation delayed neutron counting has been extensively examined by several groups of researchers whose work indicates that provided an appropriate neutron irradiation facility is available, the method is a rapid, accurate and precise analytical procedure for samples with 40 to 40×10^5 ng U (Boulanger et al., 1976).

A number of the fission products produced by thermal neutron

induced fission of ^{235}U themselves emit neutrons in their subsequent decay to stable nuclides (Table AI.1). The mean number of delayed neutrons emitted following thermal fission of ^{235}U is 0.016 neutrons per ^{235}U fission. The half-lives of the delayed neutron emitters are short (virtually all less than one minute) so that approximately 40% of the delayed neutrons are emitted within one minute of the completion of the irradiation.

Samples sealed in polyethylene capsules are stacked in a sample loader from where they are transferred pneumatically to the reactor for irradiation at pre-selected fluxes of 10^{11} - 10^{12} $n/cm^2/sec$. After irradiation, delayed neutron fluxes from the samples are counted by six BF_3 neutron detectors embedded in paraffin. After counting, the samples are ejected to a shielded storage container. The standard analytical sequence consists of a 60 sec irradiation, 10 sec delay and 60 sec count.

In practice neutron activation analysis is almost invariably performed comparatively - the signal arising from an unknown is compared with that from a standard which has undergone an identical irradiation-counting history. Ideally, sample and standards should be similar in mass, physical form, element concentration and so on. Deviations from this ideal could result in errors in accuracy. The system at the McMaster reactor centre is calibrated using IAEA reference low grade uranium ores.

The method is subject to very few interferences when used to analyze natural materials. Slowpoke reactors contain a significant fast neutron flux which can cause fission of ^{232}Th

Table A.I.1 Principal delayed neutron precursors in ^{235}U fission

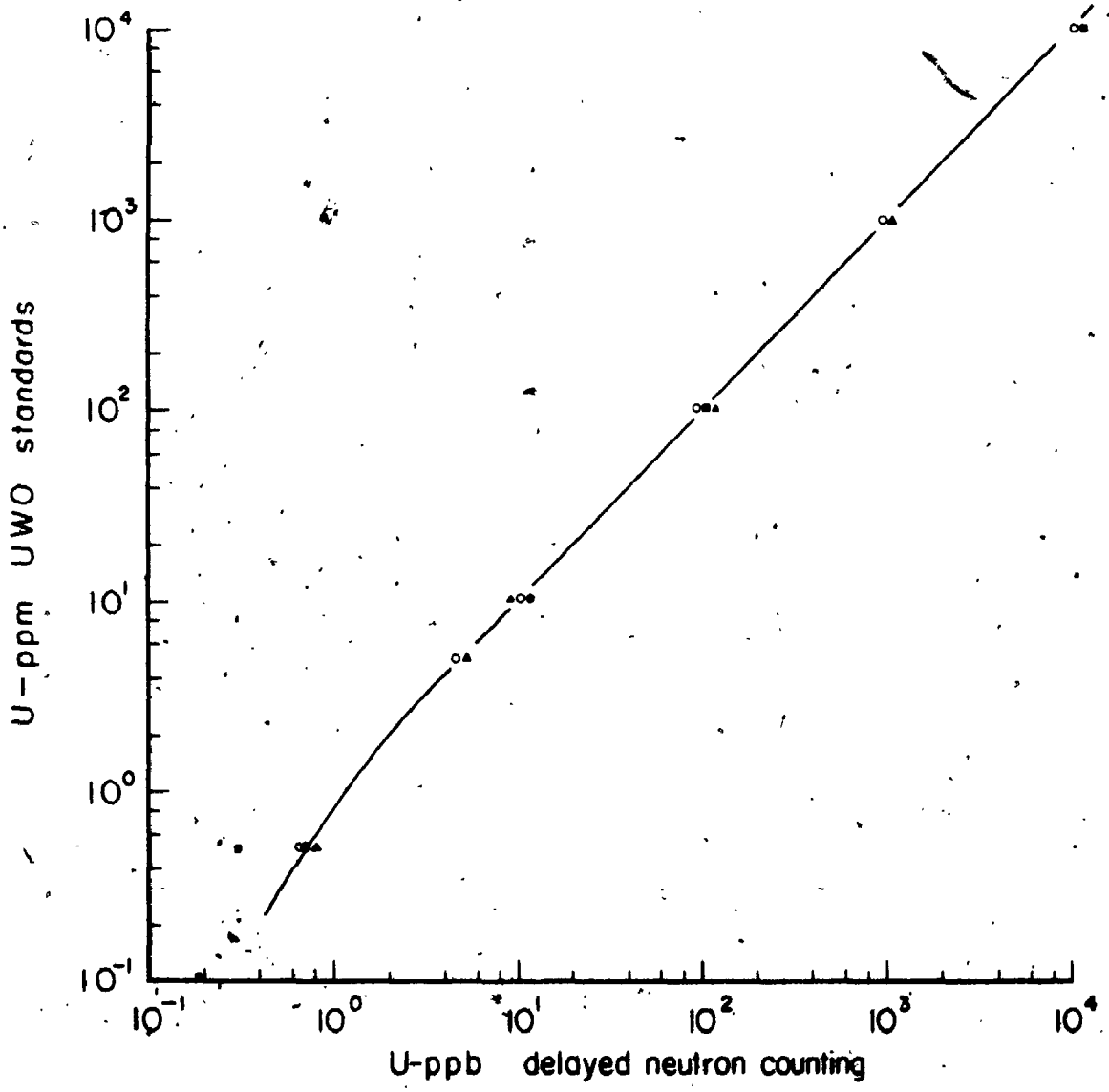
Precursor	half life (in seconds)
^{87}Br	54.5
^{137}I	24.4
^{88}Br	16.3
^{138}I	6.3
^{89}Br	4.4
$^{93,94}Rb$	~6
^{139}I	2.0
$^{90-92}Br$	1.6
^{93}Kr	~1.5
^{140}I	~0.5

data from Nucleonics, v. 20, August 1982.

Fig. AI.1 Calibration graph for 100 ml uranium aqueous standards made up at UWO, and preconcentrated by evaporation to dryness, versus their analytically determined values by means of neutron activation delayed neutron counting. Analyses conducted at the McMaster Nuclear Reactor Centre, Hamilton. Different symbols represent indivIdual sets of aqueous standards: where symbols overlap, only the extreme values are depicted.

U – calibration

neutron activation delayed neutron counting



producing delayed neutron emitters similar to ^{235}U. However the sensitivity for thorium using Slowpoke is only 1% that for uranium and aqueous thorium concentrations (0.096 ppb, Holland, 1979) are less than uranium ($\geq$ 0.6 ppb, Bloch, 1980).

Some light nuclides also decay by neutron emission- principally ^{9}Li produced by $^{9}Be(n,p)^{9}Li$ and ^{17}N produced by $^{17}O(n,p)^{17}N$. With a half-life of 0.17 sec and a 10 sec delay ^{9}Li will not cause any interference. Similarly interference from ^{17}N was experimentally shown to be negligible even though the half-life is 4.15 sec. These effects have been thoroughly examined by several authors.

Pulse build-up effects due to gamma activity, such as from the decay of $^{28}Aluminum$, is probably the most important source of interference. The counting system is electronically biased to discriminate against such gamma signals, and the quantity of Al_2O_3 required to produce interfering activity is in excess of normal levels found in geochemical samples and greatly in excess of that present in biological materials or preconcentrated waters (the range of median values reported for dissolved Al in global river waters = 250-400 ppb, Holland, 1978).

Certain other nuclides such as ^{10}B, ^{113}Cd, ^{157}Gd and indeed ^{235}U itself having large thermal neutron capture cross-sections can introduce errors due to flux depression. The effect from U can be minimized by using appropriate standards: Cd and Gd are not abundant in either most natural waters or biological materials.

Seventy five ml aliquots, split from 100 ml volumes of aqueous solutions of algae or preconcentrated waters, were placed into a polythene bag liner in a 100 ml polypropylene beaker. The beakers were loaded into an oven operating at 70°C, until the solutions had entirely evaporated, then the polythene bags were rolled up and inserted into a standard 3 ml polyethylene irradiation capsule. In this way, as much as 2000 ml river water could be concentrated into a < 3 ml volume.

The oven, which was located in a fume hood, had filtered inlet air to avoid the introduction of airborne particulates. Throughflow of air was arranged by connecting the outlet vent through two bubble bottles joined in series and filled with DIW, to a metered vacuum line. The bubble bottles served to absorb nitric acid fumes evolved from acidified waters, and to test for possible evaporative transfer of uranium during the drying process. In all instances solutions from the bubble bottles, which were themselves evaporated to dryness in the oven and analysed by delayed neutron counting, gave a response below the detection limit.

Four sets of UWO standards made up from stock solution to give aqueous uranium concentrations of 0.5, 5, 10, 100, 1,000, and 10,000 ppb were dried in the same manner as other samples, and forwarded together with unknowns for analysis. These gave a near perfect one to one correlation over four orders of magnitude in concentration (Fig. AI.1). In addition, ten litres of laboratory DIW and 100 ml HNO_3 were separately preconcentrated

and dried, to test for possible uranium contamination from reagents: results were below the limits of detection.

Fluorometric determination of uranium

This analytical technique is based on quantitative measurement of the natural fluorescence emitted by uranium. Quenching effects on uranium fluorescence by certain transition metals represents a profound interference in samples containing very low levels of uranium and unknown quantities of interfering ions. The quantitatively determined trend of quenching is in the order Cr > Fe > Cu > Ni > Zn > Ti > Al > Ca > K; 100% loss of fluorescence is experienced with Cr, 77% with Fe and 50% for Cu and Ni.

For these reasons, and given relatively high concentrations of iron in certain acid Elliot Lake waters, as well as Ni plus Co in some of the experimental series, an extraction procedure was employed in order to separate U from the interfering elements.

An aliquot of the sample is salted with aluminum nitrate [$Al(NO_3).9H_2O$] solution and the uranium is extracted into ethyl acetate ($CH_3CO_2CH_2CH_3$). The extracted sample is removed and transferred onto pellets of lithium fluoride-sodium fluoride (LiF-NaF) flux in platinum crucibles. The pellets are dried, fused and allowed to cool, forming buttons. The fluorescence of the sample is then measured using a Galvanek-Morrison fluorometer. Full details of sample preparation and measurement are given in references listed by Hart et al. (1980). All analyses were performed by Barringer Magata Ltd. of Toronto, on filtered,

Fig.AI.2 Calibration graph for uranium standards made up in DIW and TBIM separately, at UWO, versus their analytically determined values by fluorimetry. Fluorimetric analyses conducted, following an ethyl acetate extraction step, by Barringer Magenta Ltd., Toronto.

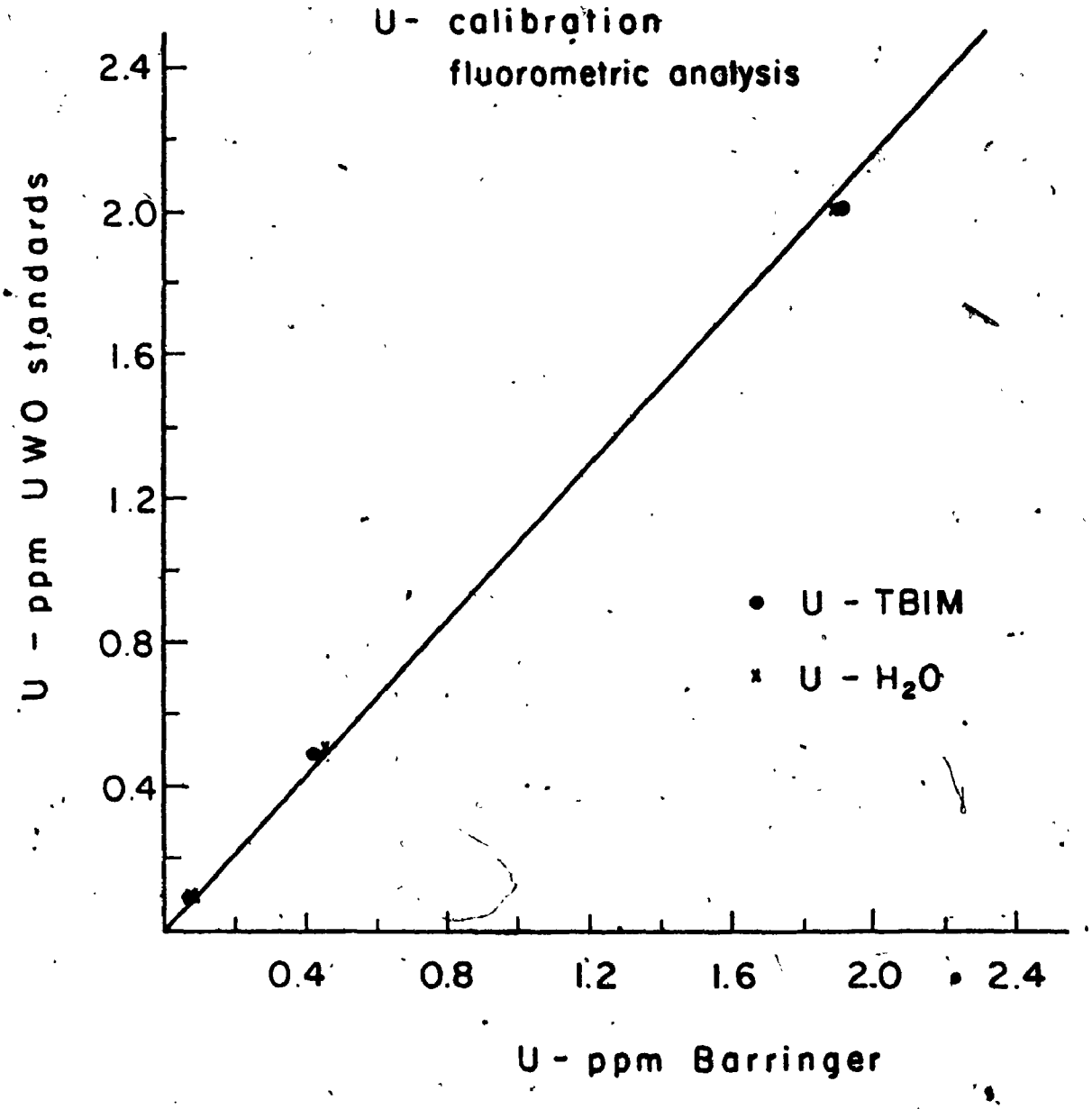

U- calibration
fluorometric analysis
U - ppm UWO standards
2.4
2.0
1.6
1.2
0.8
0.4
0.4
0.8
1.2
1.6
2.0
2.4
U - ppm Barringer
• U - TBIM
x U - H2O

Fig. AI.3 Calibration graph for aqueous uranium standards made up at UWO versus their analytically determined values, by means of fluorimetry, August, 1981. Fluorometric analyses conducted, following an ethyl acetate extraction step, by Barringer Magenta Ltd., Toronto.

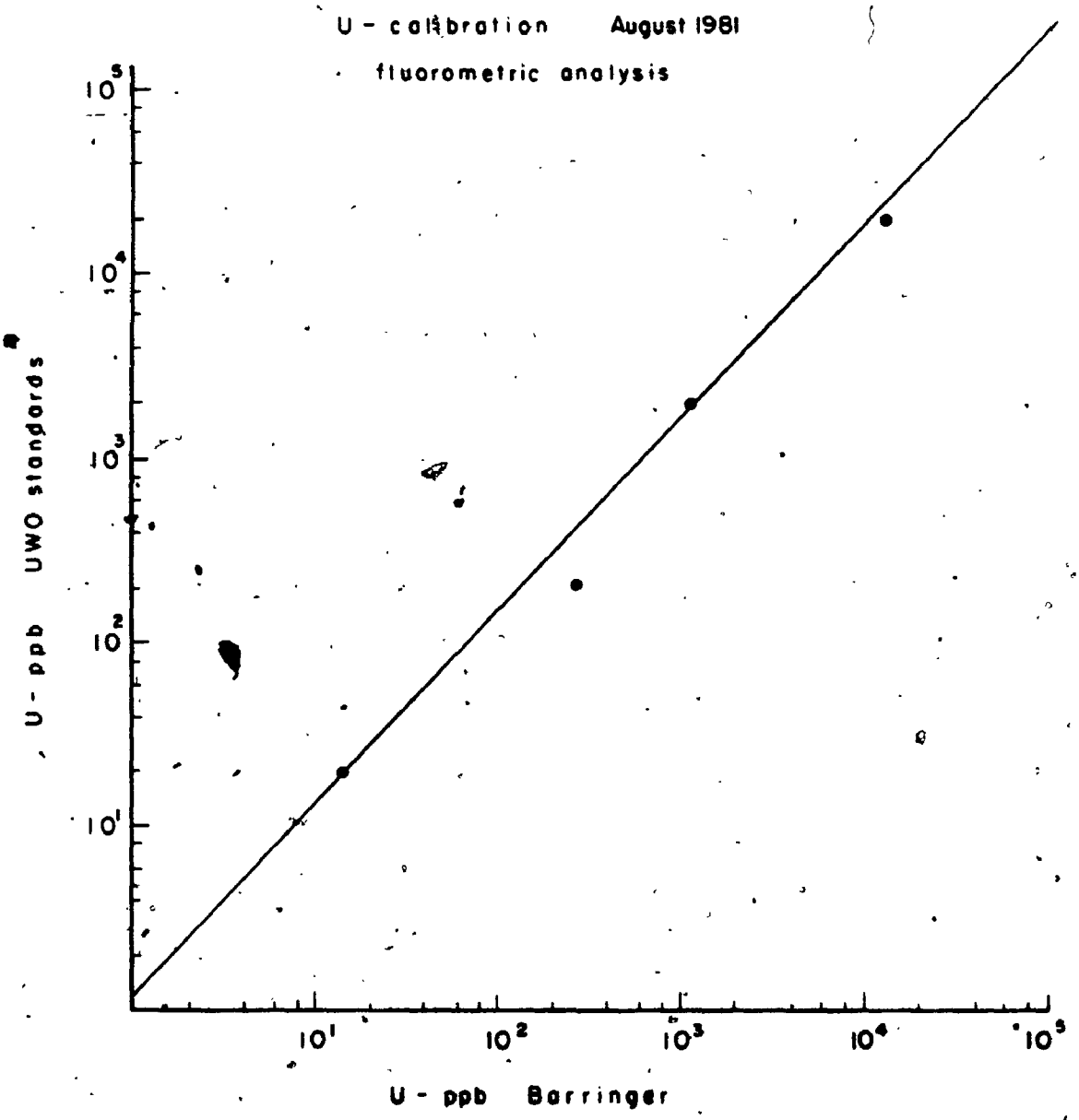

U - calibration August 1981
fluorometric analysis
U - ppb UWO standards
10^5
10^4
10^3
10^2
10^1
10^1
10^2
10^3
10^4
10^5
U - ppb Barringer

Fig. AI.4 Calibration graph for aqueous uranium standards made up at UWO versus their analytically determined values, by means of fluorimetry, September, 1982. Fluorometric analyses conducted, following an ethyl acetate extraction step, by Barringer Magenta Ltd., Toronto.

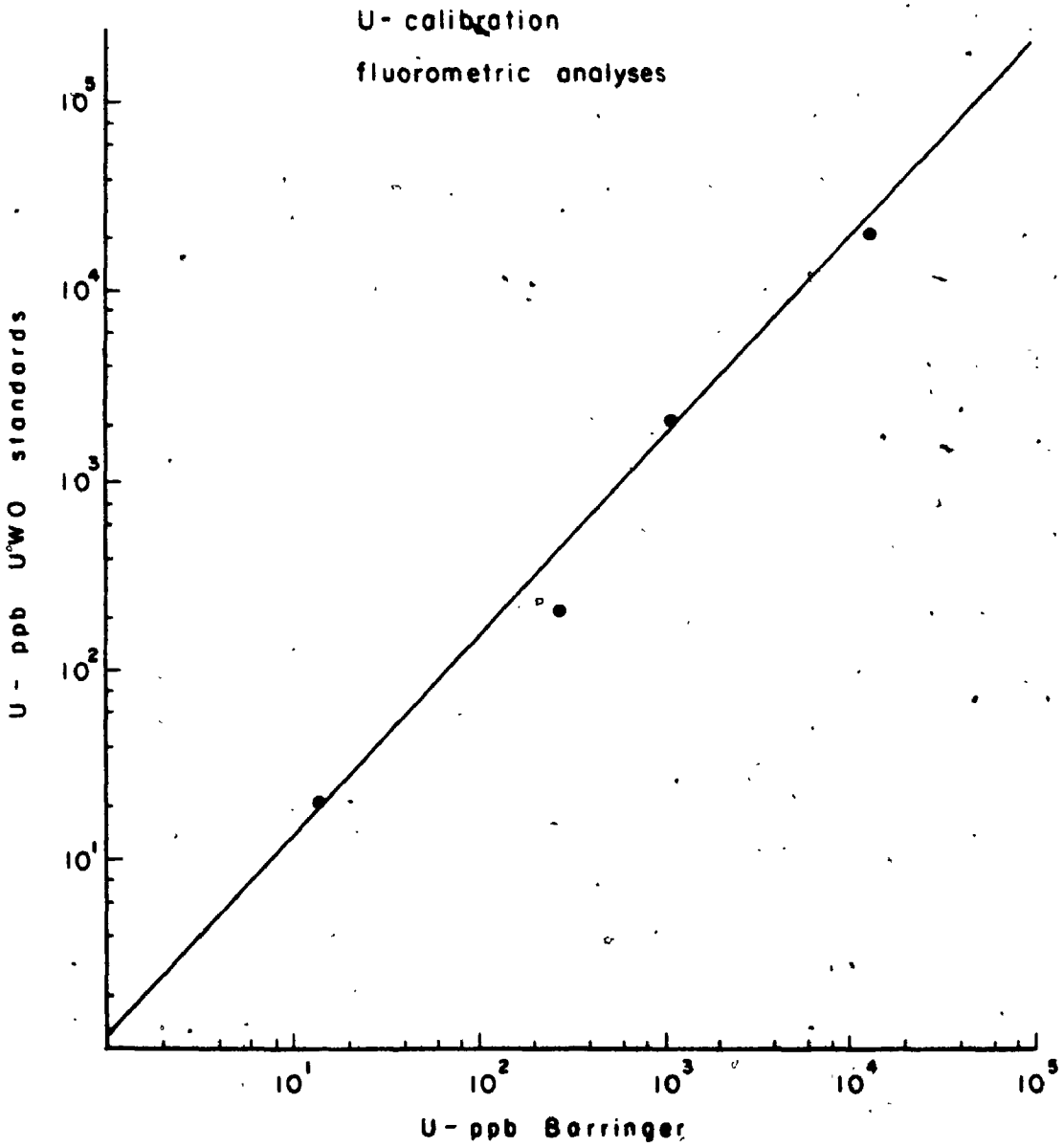
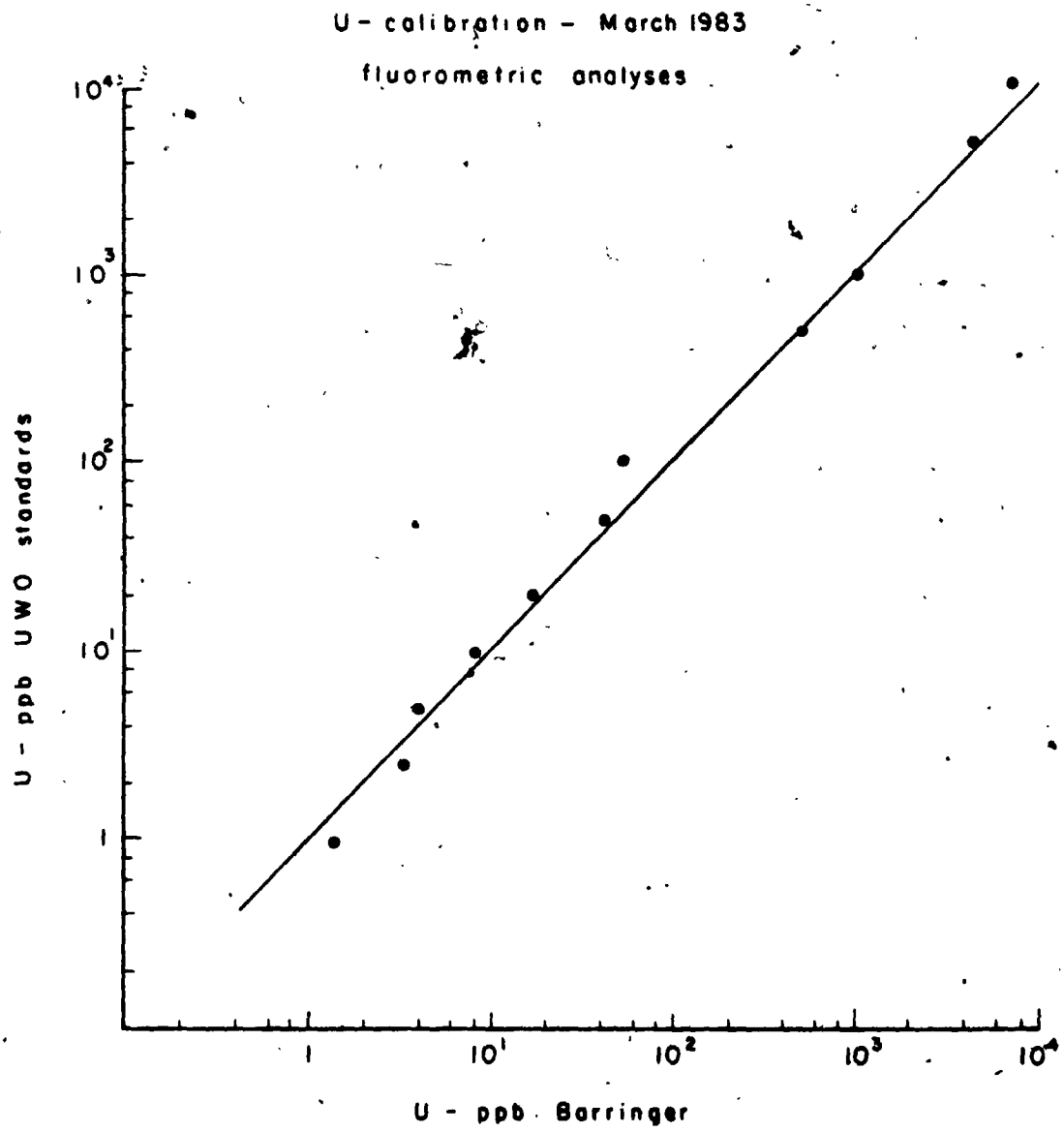

U- calibration
fluorometric analyses
U - ppb UWO standards
U - ppb Barringer
10^1
10^2
10^3
10^4
10^5

Fig. AI.5 Calibration graph for aqueous uranium standards made up at UWO versus their analytically determined values, by means of fluorimetry, March, 1983. Fluorometric analyses conducted, following an ethyl acetate extraction step, by Barringer Magenta Ltd., Toronto.


U - calibration - March 1983
fluorometric analyses
U - ppb UWO standards
U - ppb Barringer
1
10¹
10²
10³
10⁴

digested, preconcentrated and acidified samples.

Sets of aqueous standards, covering an appropriate range of concentration, and including 1, 2, 5, 10, 20, 50, 100, 500, 1000, 5000 and 10,000 ppb were forwarded for analysis along with unknowns (Figs. AI.2-5). For sets of unknowns in which TBIM was employed as a culture medium, separate sets of standards were made up in TBIM, in order to assess possible matrix effects of the culture medium. No significant differences were noted between these and standards made up in acidified DIW (Fig. A.I.2). As in the case of samples submitted for uranium measurement by delayed neutron counting, 10 litres of DIW and 100 ml of HNO_3 were separately preconcentrated to 25 ml by evaporation and analysed fluorometrically, to establish any possible reagent contamination of samples: all such results were below the limits of detection.

Determination of Ba, Co, Ni and V

Barium, Co, Ni and V were analysed by means of inductively coupled plasma emission spectroscopy (ICP). This analytical technique was selected (1) because all four elements could be measured simultaneously, (2) for the wide range of solute concentrations that can be measured, and (3) for the low levels of detection. For instance, the limits of detection are 50 ppb for Co and Ni, and 5 ppb for Ba and V.

Standards were made up in TBIM and DIW, in order to evaluate any possible matrix effect of the former in analysis of experi-

Fig. AI.6 Calibration graph for aqueous barium standards made up in TBIM and DIW separately, at UWO, versus their analytically determined values by means of inductively coupled plasma emission spectroscopy (ICP). Analyses by Barringer Magenta Ltd., Toronto.

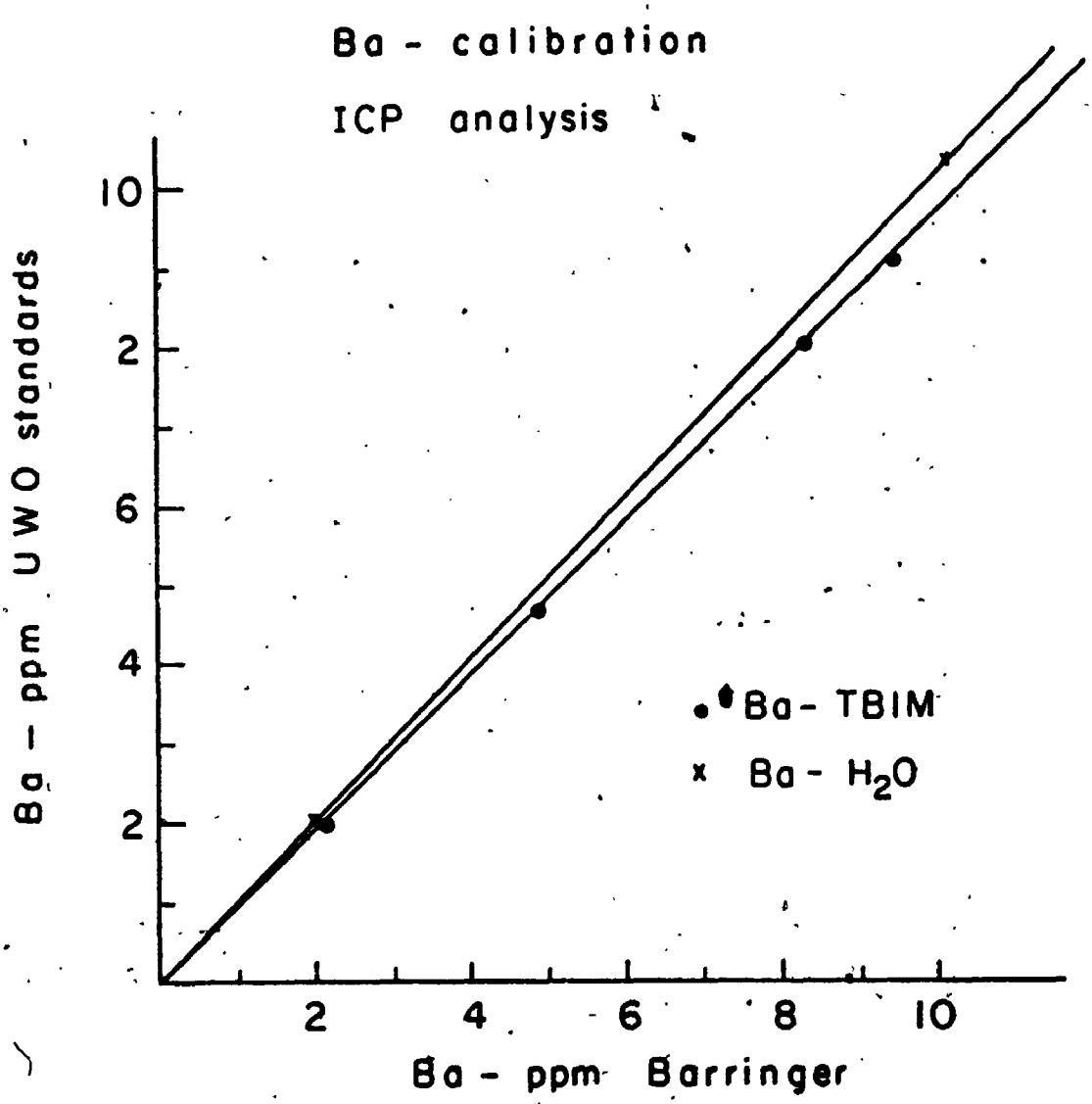

Ba - calibration
ICP analysis
Ba - ppm U W O standards
10
2
6
4
2
2
4
6
8
10
Ba - ppm Barringer
Ba - TBIM
Ba - H2O

Fig. AI.7 Calibration graph for aqueous cobalt standards made up in TBIM and DIW separately, at UWO, versus their analytically determined values by means of inductively coupled plasma emission spectroscopy (ICP). Analyses by Barringer Magenta Ltd., Toronto.

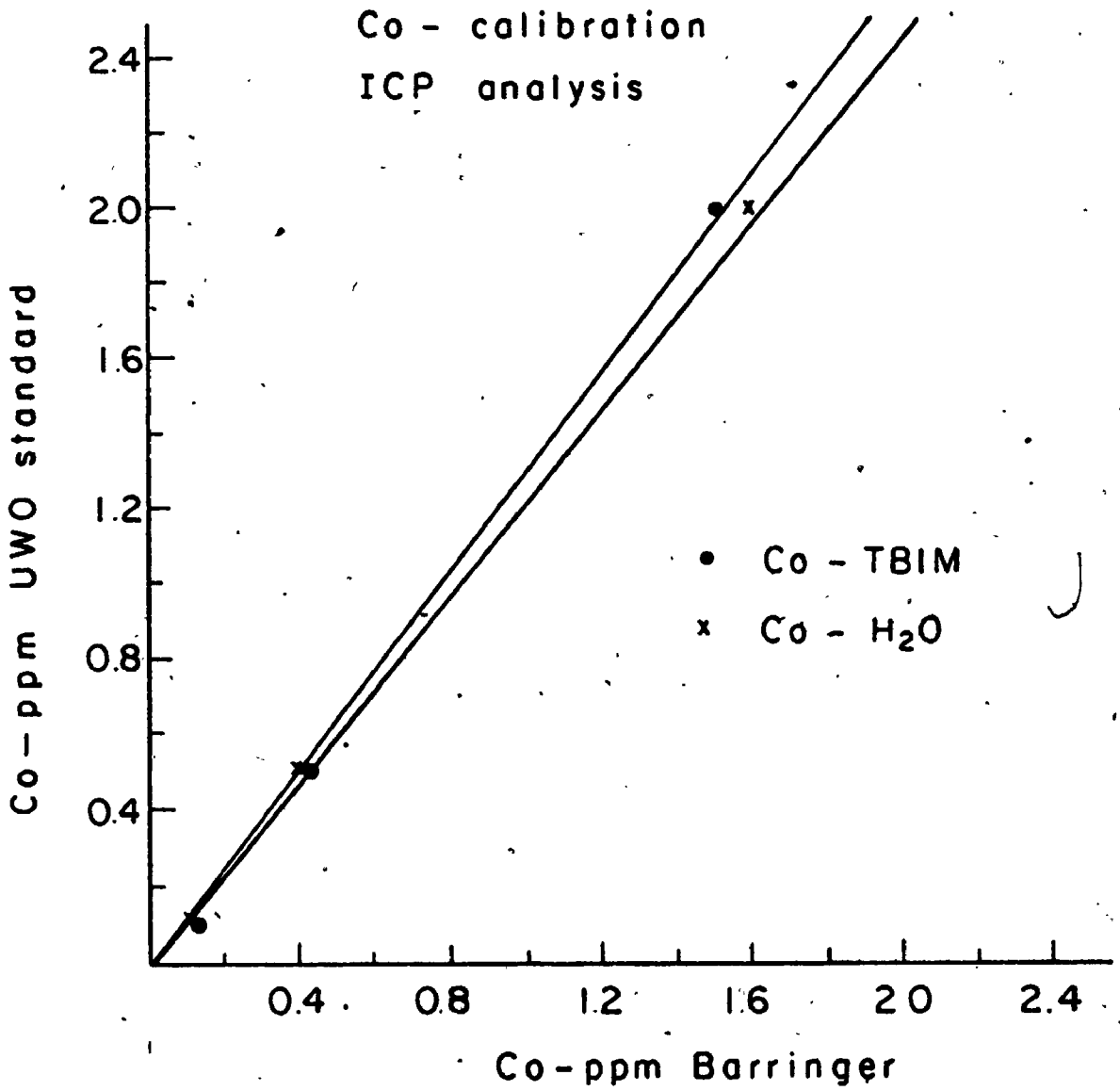

Co - calibration
ICP analysis
Co - TBIM
Co - H2O
Co-ppm UWO standard
Co-ppm Barringer
0.4
0.8
1.2
1.6
2.0
2.4

Fig. AI.8 Calibration graph for aqueous nickel standards made up in TBIM and DIW separately, at UWO, versus their analytically determined values by means of inductively coupled plasma emission spectroscopy (ICP). Analyses by Barringer Magenta Ltd., Toronto.

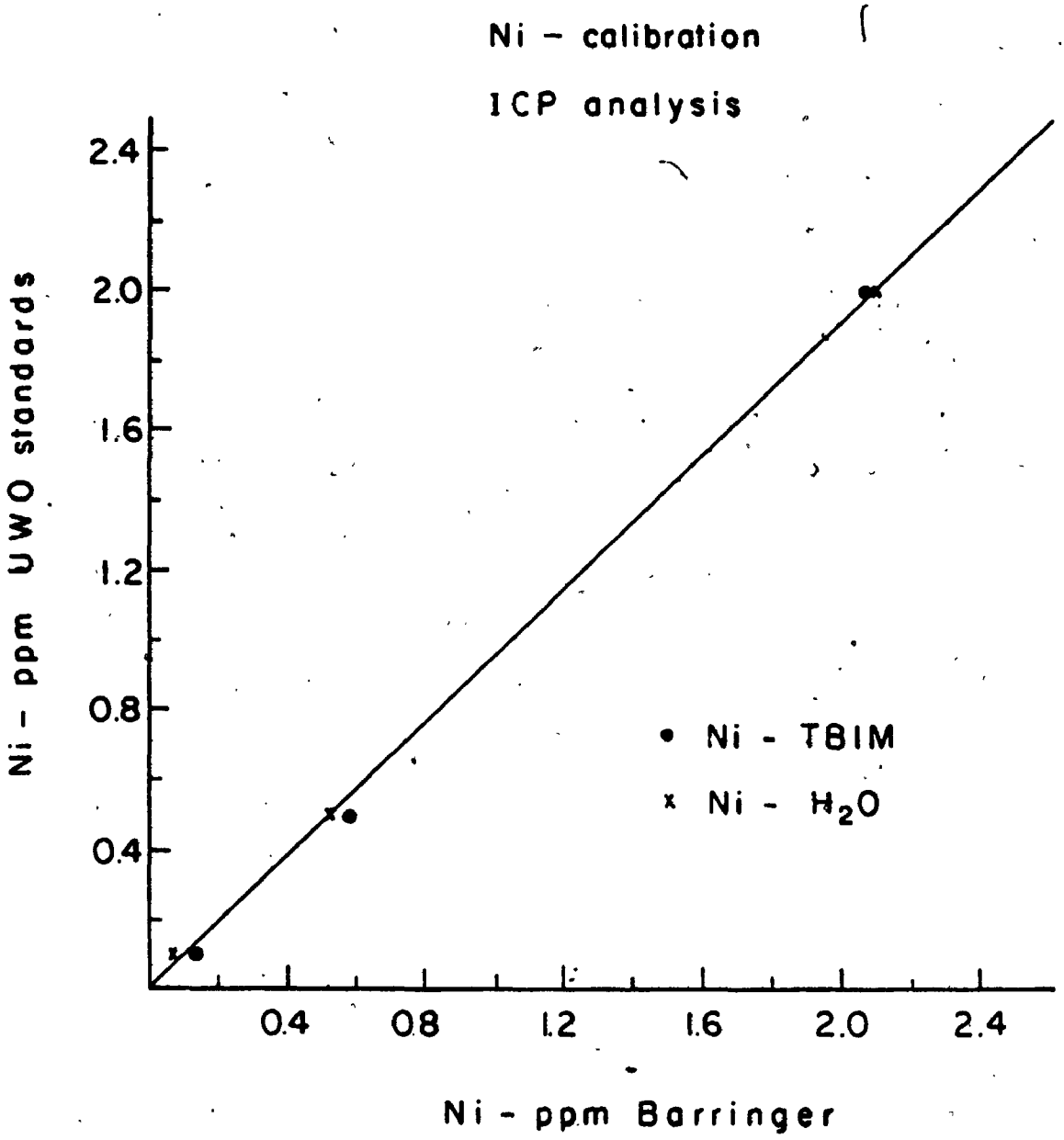
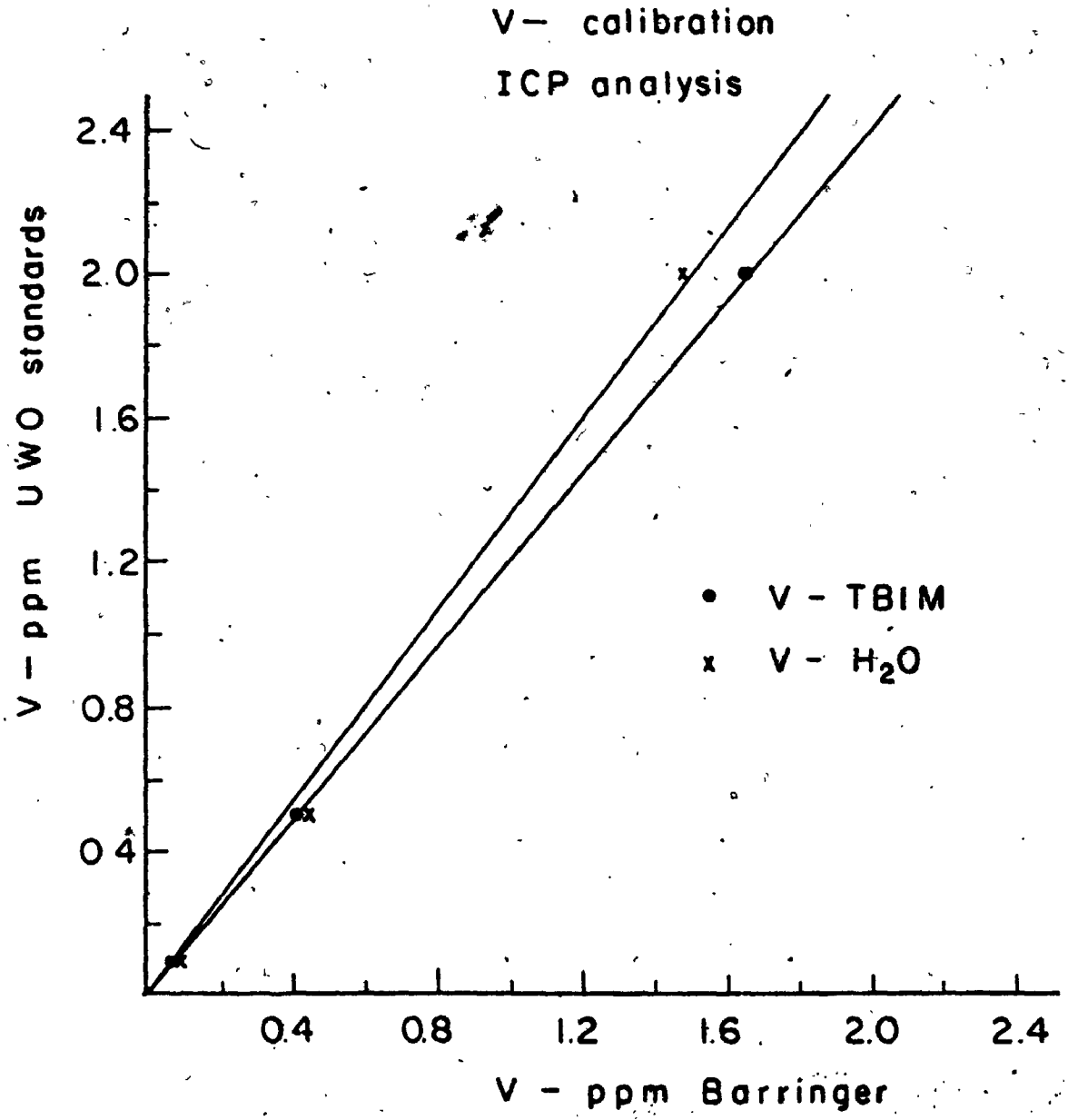

Ni – calibration
ICP analysis
Ni – ppm UWO standards
2.4
2.0
1.6
1.2
0.8
0.4
0.4
0.8
1.2
1.6
2.0
2.4
Ni - ppm Barringer
• Ni - TBIM
x Ni - H_2O

Fig. AI.9 Calibration graph for aqueous vanadium standards made up in TBIM and DIW separately, at UWO, versus their analytically determined values by means of inductively coupled plasma emission spectroscopy (ICP). Analyses by Barringer Magenta Ltd., Toronto.


V— calibration
ICP analysis
V — ppm UWO standards
2.4
2.0
1.6
1.2
0.8
0.4
0.4
0.8
1.2
1.6
2.0
2.4
V - ppm Barringer
• V - TBIM
x V - H_2O

mental solutions that contained this growth medium. Both standards and unknowns were forwarded to Barringer Magenta Ltd., Toronto for the analyses.

In general, a close one to one relationship was observed between UWO standards and their analytically determined values. The presence of TBIM appeared to induce only minor instrumental excursions compared to an aqueous matrix (Tables AI.6-9).

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